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TRAVEL OF SYNTHETIC DETERGENTS WITH PERCOLATING WATER

Stephen A. Klein, et al

California University
Berkeley, California

30 December 1962

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Second Annual Report

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SANITARY ENGINEERING RESEARCH LABORATORY
COLLEGE OF ENGINEERING
AND
SCHOOL OF PUBLIC HEALTH
UNIVERSITY OF CALIFORNIA
BERKELEY

47

TRAVEL OF SYNTHETIC DETERGENTS WITH PERCOLATING WATER

Second Annual Report

Proposal No. UCB-Eng 1618
Research Grant WP-207-C2

By

Stephen A. Klein
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Details of illustrations in this document may be better studied on microfiche

30 December 1962

Sanitary Engineering Research Laboratory
College of Engineering
and
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University of California
Berkeley

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I. INTRODUCTION

NEED FOR INVESTIGATION

The rapid rise of population in the United States, within the framework of an urban-industrial economy dependent for its very existence on the water-transport of wastes, foretells with certainty that as time goes on an ever-increasing proportion of our over-all water resource will be "used" water. But more is involved than mere numbers of people exerting an increasing per capita demand for water. Each year the variety of heretofore unknown waste materials is multiplied by our industrial activities. Hence, the time approaches when the mass of used water can no longer be prevented from becoming critical. In many cases this problem can be overcome only by upgrading the quality of used water before permitting it to mingle with new hydrological waters. Alarm has consequently been rising over the presence of refractory materials in waste waters; and of these intractable materials, alkylbenzenesulfonate (ABS) has been given the greatest publicity and has been the object of most investigative work.

Because ABS has not been shown to constitute a health hazard, mere vexation with some of its properties is not sufficient reason to prohibit its manufacture and sale for household cleansing. Therefore, in spite of a growing public opinion that such an eventuality may come about, research on the behavior of ABS in all aspects of the water environment is still of major importance. In no other manner will it be possible to assess the feasibility of removing ABS from waste water to avoid drastic prohibitory action. Research on methods of removal of other refractory materials might conceivably find ABS in sewage to be the economical frothing agent necessary for their removal.

In the western United States the fate of ABS in soils is of more than academic importance. Direct reuse of reclaimed waste waters in irrigated agriculture is a current practice in certain semi-arid areas, and undoubtedly will become more widespread in the future. Substitution of sewage or sewage effluents for the normal source of irrigation water, however, poses the question of whether the value of the water in sewage and sewage effluents is in any way decreased by its contaminants. The value of sewage as a fertilizer has been recognized throughout the ages, but today with its content of chemically synthesized substances this value may be somewhat diminished. It is important to the grower to avoid reducing crop output by even a few per cent. The persistence of ABS during sewage treatment, therefore, gives a degree of urgency to studies on the interrelationships of ABS and soils, and on the effects of such detergents upon plant growth, as well as on the uptake of detergents by plants (especially the edible portions).

NATURE AND SCOPE OF INVESTIGATION

The results herein reported were derived from investigative work completed by the authors during the period 1 October 1961 to 30 September 1962--the second year of a three-year investigation of the "Travel of Synthetic Detergents with Percolating Water." The investigation is being conducted within the Sanitary Engineering Research Laboratory of the University of California under the terms of Research Grant WP-207 awarded by the National Institutes of Health to the Regents of the University on the basis of proposals UCB-Eng-1409 and UCB-Eng-1618. The principal objectives of the investigation as stated in the proposals are:

1. To seek an economical means of de-aerating and disposing of froth in which the detergent component of sewage has purposefully been concentrated by a process of induced frothing previously developed by the project investigators.

2. To determine the degree of detergent removal by adsorption and other phenomena in both the biologically active and inactive strata of pervious soils or water-bearing strata when sewage effluents are reclaimed by ground water recharge through surface spreading or direct injection.

3. To study the effect of detergents upon field plants typical of those irrigated, or proposed for irrigation with sewage effluents in the Southwest, together with the effect such vegetation may have in reducing the detergent content of percolating effluent or in concentrating the detergent in the plant.

The second of the three objectives was given first priority and the results of experiments directed to it were presented in the First Annual Report, dated 30 December 1961 [1]. In this first year of study a series of laboratory-scale experiments were conducted on five types of agricultural soils common in California and on aquifer material from the University of California's experimental recharge-well field. All of these were concerned with the adsorption of ABS on soil particles, and with other phenomena which might lead to a decrease in detergent concentration during passage of ABS-bearing water through the soil mantle or through a water-bearing stratum. Studies were made with 25-cm-long soil columns loaded with a feed solution containing various ions normal to sewage effluents, together with ABS and tracer amounts of ABS^{35}. Individual experiments involved sterile and biologically active soil columns under saturated and unsaturated flow conditions.

The results of these experiments supported the following conclusions:

1. All soils examined displayed an ability to adsorb ABS. Adsorption or retention was greatest in the top zone, probably as a result of selective entrapment of the larger ABS molecules. (ABS used in the investigation was made from alkylbenzene having a range of from 12 to 16 carbons in the alkyl group.)

2. All soils showed a tendency for continued adsorption of ABS during periods of static contact between soil and ABS-bearing liquid.

3. The most adsorptive soils were those with the most uniform distribution of particle sizes.

4. In soil columns 25-cm deep and 2.5-cm in diameter, the most adsorptive soils removed 50 per cent of the ABS present in the first liter of liquid percolating through it. (The range for various soils was from 66 to 22 per cent removal.) After 5 liters, only about 5 per cent of the influent ABS was removed.

5. All soils exhibited an early breakthrough of ABS^{35}, the more permeable soils (least adsorptive) requiring the least period of time. The ratio of effluent to influent ABS reached 90 per cent in about one-half the time required for 100 per cent breakthrough.

6. There is virtually no difference between the biologically active and biologically inactive soils in their ability to adsorb ABS under conditions of either saturated or unsaturated flow.

7. Some bio-degradation of ABS occurred during periods of resting of biologically-active unsaturated soil, but the rate was too small to be of consequence in preventing breakthrough of infiltrate ABS to ground water, the rate of buildup to the limit of adsorption on soil being hundreds of times that of reduction by bio-degradation. Degradation was confined largely to the top one inch of the soil column.

8. Aquifer sand from the Richmond Field Station recharge-well field adsorbed ABS in amounts similar to that found for Oakley sand, the least adsorptive of the five agricultural soils utilized.

9. In the range of concentrations of ABS used in the experiments (0.001 to 10.0 mg/l), adsorption was directly proportional to the ABS concentration in the infiltrating liquid.

10. ABS adsorbed on soil was freely exchanged with ABS in the percolating liquid in a manner similar to the Langmuir adsorption isotherm, in which gas molecules are continuously adsorbed and desorbed from a solid surface.

11. Eighty per cent of adsorbed ABS was readily leached from the soil by detergent-free water. Continual application of leaching water produced a further slow reduction in ABS content of the soil, down to some minimum residual value.

12. The distribution of ABS throughout the soil column indicated that molecular entrapment between particles of clay was responsible for that part of residual ABS retained after leaching--the greatest retention occurring at the surface zone with uniform retention at lower horizons.

13. Analysis of soil by zones demonstrated that below the surface zone (abnormal for reasons of selective molecular entrapment of ABS) adsorption of ABS with depth conformed closely to the plate theory, which postulates that the solute adsorbed by soil at any horizon is proportional to the concentration of the solute reaching that horizon.

14. The degree of adsorption of ABS on soil, while appreciable in amount, was not sufficient to prevent an ABS breakthrough in a short period of time relative to that needed for waste disposal projects. Furthermore, adsorbed ABS could be expected to continue to reach ground water in decreasing amounts after application of ABS was discontinued.

15. Under equilibrium conditions the ABS content of a ground water might be used as a good index to the degree of recycling of waste water which occurs in any individual case.

16. The results of the experiments reported conformed with what might be expected on the basis of theoretical concepts of adsorption phenomena and known characteristics of anionic detergents.

Further attention to the bio-degradation of ABS in soils was deemed necessary. This (Second Annual) report, therefore, presents the results of experiments with methods of improving rate of ABS degradation by varying soil-loading rates and frequency of application, and by utilizing soil stratification. Principally, however, the report deals with investigative work directed to the third objective--the effect of ABS on plant growth, and the degree of uptake of this detergent by growing plants.

Standard test plants--sunflowers, barley, and Lupinus albus--were used in experimental work instead of typical irrigated field crops such as alfalfa, cotton, sugar beet, etc. because more is known of the former group's behavior under laboratory conditions. These studies were designed to provide some answer to each of the following questions:

1. Is ABS toxic to plants, and if so, at what concentration?

2. Does toxicity vary for different plant species?

3. Does ABS accumulate in certain parts of the plant, particularly the edible portion?

4. To what extent is ABS degraded in the plant structure?

5. Is ABS toxicity, if any, masked or outweighed by the nutrient value of sewage?

Toxicity studies were conducted with plants grown rapidly in a water culture as well as with those rooted normally in agricultural soils. In the latter case both fertilized and unfertilized soils,

irrigated with fresh water and with primary sewage effluent, were used to weigh the nutrient value of sewage against any toxicity of the ABS content. Long-term studies with barley permitted maturation of the seed as well as the body of the plant for study of concentration and degradation of ABS.

II. THE INVESTIGATION

INTRODUCTION

Experiments concerned with maximizing the ABS-degradation capabilities of soils involved laboratory-scale column studies. These studies were carried out on feed solutions equivalent to a secondary sewage effluent containing approximately 20 mg/l BOD, in addition to ions normal to sewage effluents. ABS concentrations of the order of 1 mg/l were traced with ABS^{35}.

Work dealing with the effect of ABS on plant growth, and the uptake of ABS by plants, was carried out in water culture and soil studies. Two species of plants (sunflower and barley) were grown in water culture at ABS concentrations of 0, 10, and 40 mg/l, and three species (sunflower, barley, and Lupinus albus) were grown in soil at ABS concentrations up to 50 mg/l. In soil studies on both fertilized and unfertilized soils, water solutions of ABS were compared to primary sewage effluents containing added amounts of ABS. Corn and Lupinus albus seeds were germinated in the presence of various concentrations of ABS to determine ABS effects at this stage of growth.

MATERIALS AND METHODS

Degradation in Soils

Technique and apparatus used for studies of ABS degradation in soil columns have been previously described in the First Annual Report [1].

Plant Growth in Water Culture

The water culture method of Hoagland and Arnon [2] was employed in plant growth studies. Plants were grown in a nutrient solution (cf. Appendix A) in 20-liter containers fitted with air lines. Continuous aeration provided oxygen to plant roots and maintained a well-mixed solution. The apparatus is shown in Figure 1, together with platform and structures for supporting plants.

Seedlings germinated and grown in vermiculite for approximately one week (Figure 2) were supported over the metal containers in corks fitted in the wooden platforms. A dacron-wool wrapping at the base of the stems secured seedlings in the corks and yet provided sufficient flexibility to accommodate plant growth. Plants were grown at room temperature in artificial light provided by a bank of fluorescent lights which were turned on for 16 hours each day by an automatic timer.

Five plants were grown in each of the nine containers. This provided 15 plants for each of three ABS concentrations tested. The

FIGURE 1. EQUIPMENT USED FOR GROWING PLANTS IN WATER CULTURE

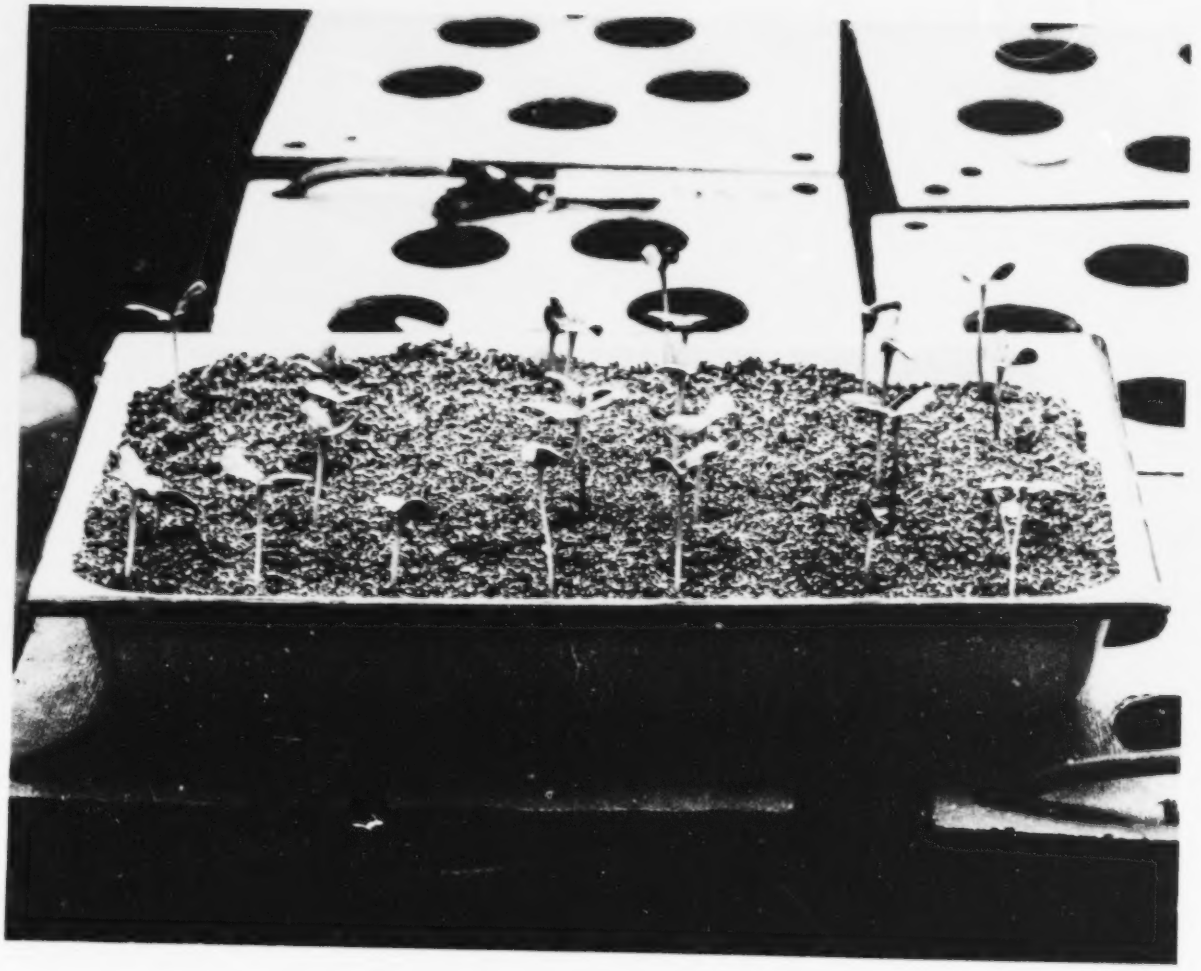

FIGURE 2. WEEK-OLD SUNFLOWER SEEDLINGS GERMINATED IN VERMICULITE

position of the individual containers under the light bank was randomly determined by the magic square method.

Tracer ABS^{35} was added to the water culture along with the carrier ABS, in order that a measurement of the plant's uptake of ABS could be made by radioassay.

Plant Growth in Soil

Nursery flower pots, 6 inches and 7 inches in diameter, were used in soil studies of plant growth. Good soil drainage was insured by using a porous soil, Yolo sandy loam, and an underdrain of Fairoaks pea gravel overlaid by Monterey coarse and Monterey fine sand.

In unfertilized soil experiments, tap water was used for irrigation of plants. To prevent buildup of salt concentration in the soil, pots were flushed with excess water.

When fertilization of the soil was required, chemical fertilizers were added in accordance with the method of J. Vlamis [3]. These chemicals and their concentrations are given in Appendix A. Loss of water and nutrients was prevented by saucers placed under the pots to receive the excess. Plant roots then drew the excess back up into the pots. To avoid buildup of salt concentration in the soil, plants were watered with distilled water.

Radioassay of Plants

After the fresh weight of plants was taken, the dry weight was obtained by oven-drying at 105°C for one hour. The dried plant was ground to a fine powder with a mortar and pestle, packed in a planchet, and counted to determine total S^{35} activity. Then the powdered plant was extracted twice with 100 ml of 70 per cent ethanol for one hour on a steam bath. The standard ether extraction for ABS^{35} was made on the ethanol extract with the water phase analyzed for degradation to $S^{35}O_4$ by the $BaSO_4$ test. In some instances the ether extract was adsorbed as usual on activated carbon, but when sufficient plant material carried over into the ether phase this material was used as the adsorbing media instead of the carbon. Self-adsorption studies (cf. Appendix B) were performed on each media to relate all activity to a weightless basis.

Seed Germination

The method employed was developed by F. Murayama [4] for testing pharmacological products. Lupinus albus seeds were soaked in distilled water in the dark for 24 hours. The swollen seeds were then planted in vermiculite flats and incubated in a dark water-saturated incubator at 20°C for 2-1/2 days. By this time the seeds had put out a root which had a hypocotyl length of about 15-35 mm. The hypocotyl was clearly defined and it was easy to measure its length with a millimeter scale.

Seeds with hypocotyl lengths between 21-30 mm were selected for the experiment. A yield of between 40-70 per cent of seeds of the desired hypocotyl length was obtained. All transfer operations and test incubations were carried out in artificial light to prevent photosynthesis.

One-quarter strength Hoagland's Solution [5] was made up (cf. Appendix A), and the desired amount of ABS added. The solution was aerated by vigorous shaking, then poured into small test tubes. There were 35 tubes at each ABS concentration. The seedlings were withdrawn from the vermiculite, washed in distilled water, measured, and placed in the small test tubes so that the complete hypocotyl was immersed in the test solution. The hypocotyl lengths of each group of seedlings was matched so that their average initial lengths fell within 0.1 mm.

III. EXPERIMENTAL RESULTS

BIOLOGICALLY-ACTIVE SOIL COLUMNS

Introduction

Efforts to improve the degradation of ABS in soil columns involved two studies. In the first, simulated sewage effluent was applied in irregular and infrequent large-volume doses, and then on a regular short-term cycle of dosing and resting. The second study was conducted on a column of relatively coarse soil overlaid by a finer soil to restrict percolation rate and insure aerobic conditions in the underlying coarse soil.

Degradation of ABS in Hanford Soil

A 25-cm column of Hanford sandy loam was seeded with settled sewage (filtered with glass wool) for a period of nine days. Between each day's dose of sewage, the soil column was drained by applying suction to the effluent tube. Continuous aeration of the sewage entering the soil column maintained aerobic conditions during percolation.

Simulated sewage effluent containing 1 mg/l ABS, tracer ABS^{35}, and 20 mg/l BOD was introduced into the seeded column and continuously percolated until a 100 per cent breakthrough of S^{35} and ABS^{35} was obtained, requiring 2.9 liters (Figure 3). The column was then operated on a "percolation with static contact schedule" for 33 days. During this period the average loading rate was 1.1 mgd/acre, inclusive of static contact periods. The average breakdown to SO_4 and intermediate products was 25 per cent.

After 7-liter throughput, a dosing siphon was installed to deliver 35 ml feed to the column every six hours, resulting in a regular schedule of one hour of percolation and five hours of resting. This produced an increase in ABS breakdown to 35 per cent, but at a decreased loading rate of 0.29 mgd/acre. Thus, a decrease in over-all loading rate of about 72 per cent resulted in only an additional 10 per cent ABS degradation, but complete degradation to SO_4 increased appreciably from 9.3 per cent to 31.4 per cent.

Degradation of ABS in Hanford-Oakley Soil Column

An investigation was made to determine the effect on ABS degradation of a coarse soil overlaid by a stratum of fine soil to simulate natural soil stratification as it frequently exists in the field. The limitation on percolation rate imposed by the fine soil prevented saturation of the lower soil strata during continuous percolation and therefore represented an ideal natural situation for ABS degradation.

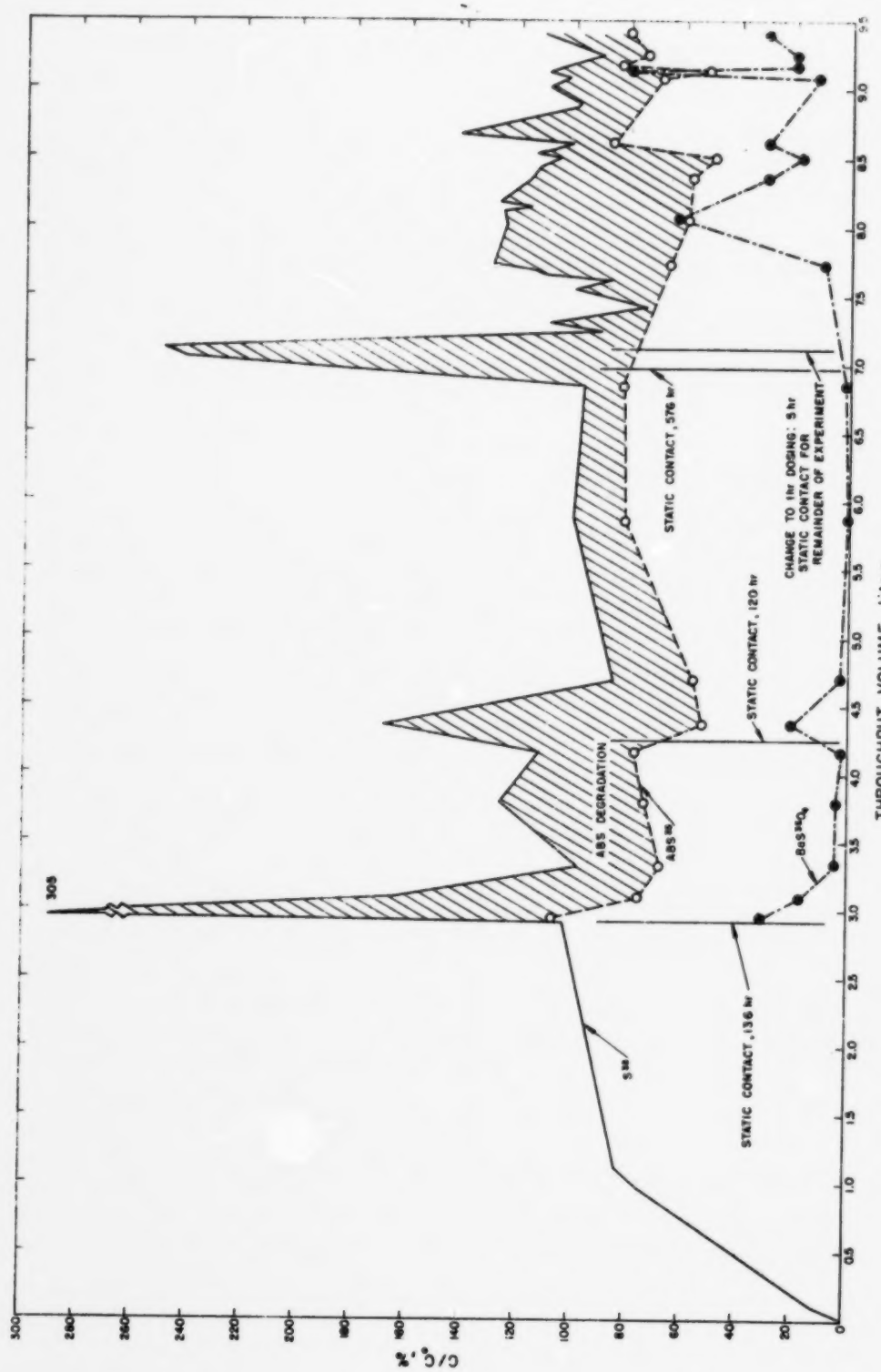

FIGURE 3. DEGRADATION OF ABS^{35}(ABS) PERCOLATED THROUGH UNSATURATED, BIOLOGICALLY-ACTIVE HANFORD SOIL COLUMN

The experimental apparatus consisted of two 2.5 cm diameter columns consisting of a 14-cm long column of Hanford fine sandy loam positioned above and connected by rubber tubing to a 94-cm long column of Oakley sand.

A 10 per cent sewage solution was percolated through a column for one week. Following this, 6.7 liters of sterile simulated sewage effluent containing 1 mg/l ABS, tracer ABS^{35}, and approximately 20 mg/l BOD was percolated through the columns over a 24-day period. It was not possible to maintain sterile conditions in the feed solution, so in order to retard ABS breakdown in the feed reservoir, another solution was prepared which contained no nutrient broth or phosphate. Over a seventeen-day period 5.4 liters of this solution was percolated continuously through the column. Throughout the experiment the liquid entering the column of Hanford soil was aerated to insure aerobic conditions.

A complete breakthrough of S^{35} activity was not achieved. Figure 4 shows that after 8 liters throughput there was only a 70 per cent breakthrough which subsequently increased to 91 per cent at 12 liters throughput. For the last two liters throughput, average total activity breakthrough was 84.3 per cent and ABS breakthrough averaged 67.1 per cent. Thus, ABS degradation, the difference between these two figures, was 17.2 per cent. There was a noticeable trend of increasing degradation at the end of the experiment. During the final liter, throughput average ABS degradation rose to 20.5 per cent. Complete degradation to sulfate for this period averaged only 2.3 per cent.

Comparison of this study with the previous one [1] shows a decrease in loading rate of 38.5 per cent (from 1.1 mgd/acre to 0.65 mgd/acre) resulting in a 4.5 per cent decrease in ABS degradation, which indicates that intermittent dosing is preferable.

EFFECT OF ABS ON PLANT GROWTH

Introduction

Because of rapid growth, experiments on ABS uptake and movement in plants were first carried out using the water culture method commonly employed in toxocological studies. Subsequently, the experiments were repeated with plants rooted in normal agricultural soil. Two plant species, the dicotyledon (sunflower) and the monocotyledon (barley), were chosen for these studies because of their rapid growth and resistance to insects and disease. Included in soil studies was a third species, Lupinus albus (a dicotyledon). Lupinus albus seeds were later used, together with corn seeds, in studies of the effect of ABS on germination.

Plant Growth in Water Culture

Sunflower Experiments. In the first experiment sunflowers were grown in nutrient solutions containing 0, 10, and 40 mg/l ABS for a period of 12 days. There were five plants per container and three replicate

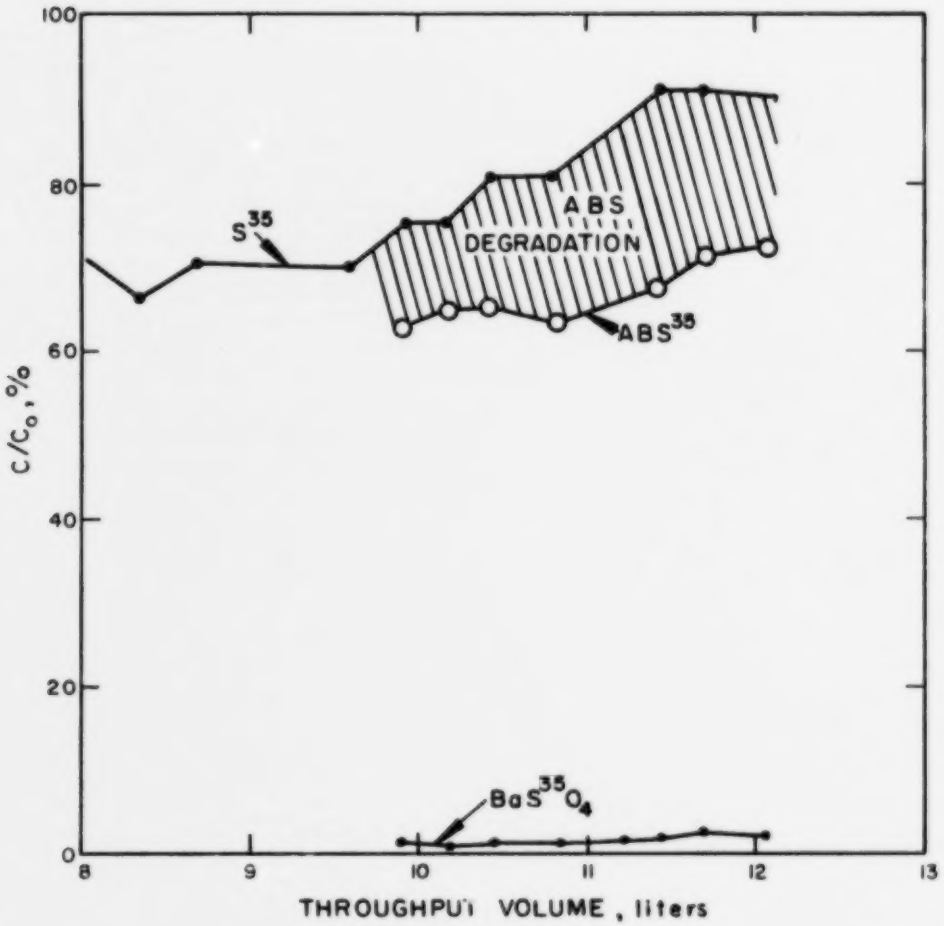

FIGURE 4. ABS DEGRADATION IN STRATIFIED HANFORD-OAKLEY SOIL COLUMN

containers per concentration, i.e., a total of 15 plants grown at each concentration. Foam depressant added to the containers successfully controlled foaming caused by continuous aeration. Plant growth was estimated by measuring leaf length and epicotyl length at intervals throughout the experiment. At the end of the growth period, the roots and stems were treated separately. Special care was taken to wash off the nutrient solution from the roots with distilled water.

After only five days in the water culture, it was apparent that ABS had a toxic effect on the plants (Figures 5, 6, and 7). After 12 days' growth, the average length of the first leaves of the control plants was almost double that of the 10-mg/l plants and 4.5 times the 40 mg/l plants (Figure 8). Similar inhibition of epicotyl growth is shown in Figure 9. Photographs taken after 12 days of growth (Figures 10, 11, and 12) illustrate the retardation produced by ABS at the conclusion of the experiment. In these photographs it is noticeable that the control plants have lush roots and foliage whereas the plants grown in 40 mg/l ABS appear particularly retarded, and even at 10 mg/l ABS a marked growth inhibition occurs. The extent of inhibition is shown by final plant weights (Table I). The mean root weight of the control plants is 7 times that of the 10-mg/l plants, and 84 times that of the 40-mg/l plants. Similarly, the control mean weight of the stems and leaves is 3 times and 9 times greater, respectively; or by taking the plant as a whole, the control is 4 times and 12.5 times greater. Water content does not vary radically in the stems of the three groups, but is drastically reduced in the roots--dropping from 91.1 per cent in the controls to 64.2 per cent in the 40-mg/l group.

Not only were growth differences apparent, but, as may be seen in Figure 13, the leaves of the 10-mg/l plants were extremely chlorotic. A yellowing of new leaves and of older leaves near the stems indicated that even at fairly low concentrations, ABS can cause chlorosis.

Radioassay of the test plants at the conclusion of the experiment revealed that ABS was concentrated in the roots but was also present to a lesser extent in the stems and leaves. Results are presented fully in Table II, and in summary form in Table III. The ABS values shown in these tables are obtained by conversion of the measured ABS^{35} to total ABS using the known tracer-to-carrier ratio of the liquid. The S values are derived from the measured S^{35}, or total activity, and converted in the same manner. S values thus refer to all sulfur products derived from the carrier and tracer, and therefore include degradation products of ABS, as well as any unaltered ABS. Plant uptake of ABS increased with increasing ABS concentration in the water culture. This increase is not proportional, however, since a four-fold liquid ABS change from 10 to 40 mg/l produced a 22-fold increase in root ABS concentration and a 37-fold increase in stem and leaf ABS concentration. In the 40 mg/l group, roots concentrated ABS to over 9 per cent on a dry weight basis, and the average plant root contained over one gram of ABS. In this group the stems and leaves concentrated ABS to approximately 0.2 per cent of the dry weight, and the average plant contained almost 0.2 g ABS. At 10 mg/l plant roots concentrated ABS to over 0.4 per cent and the average plant root contained slightly more than 0.2 g ABS. Stems and leaves of this group concentrated ABS to less than 0.01 per cent dry weight and the average plant contained only 14 µg ABS in the stems and leaves.

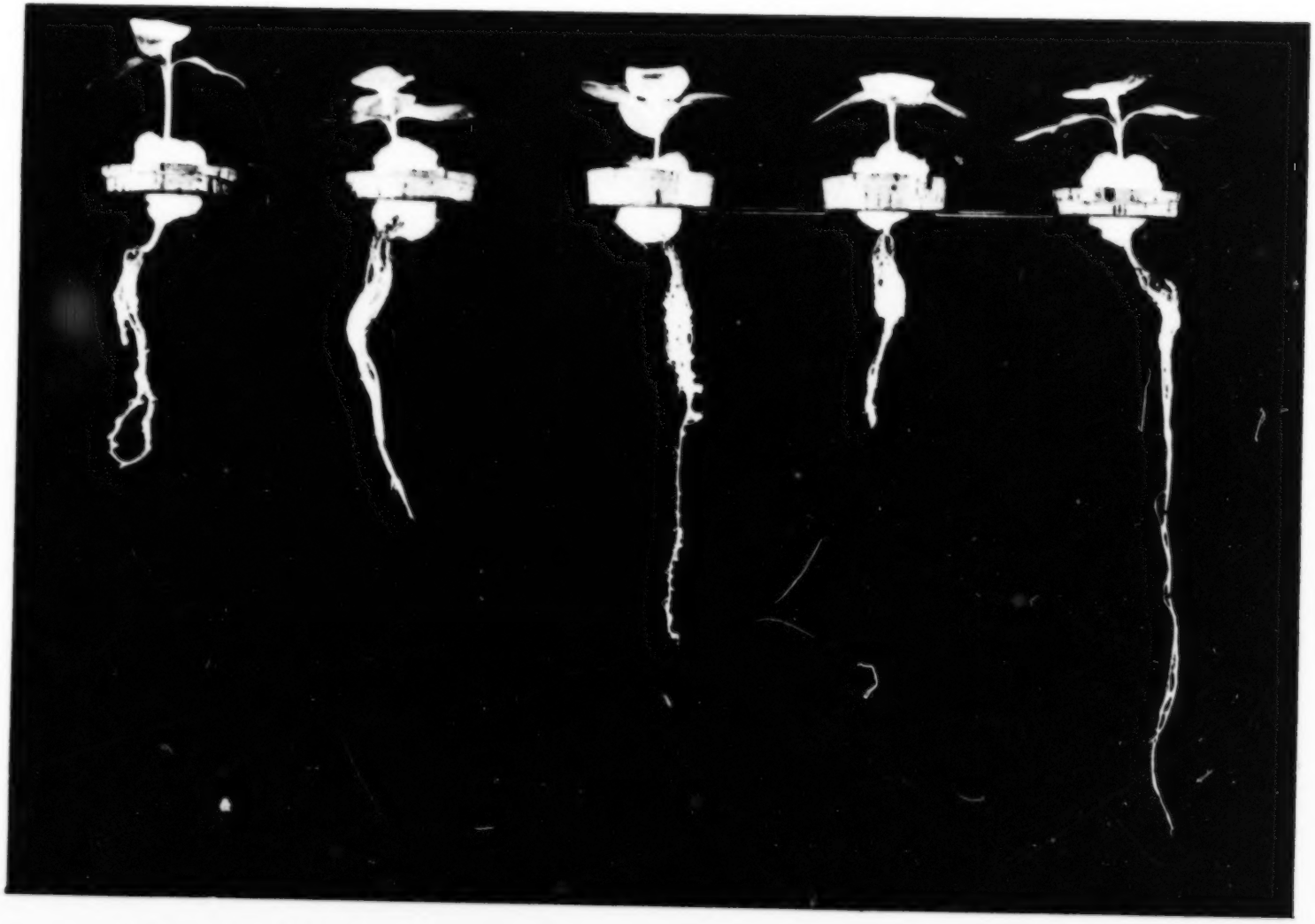

FIGURE 5. SUNFLOWERS GROWN 5 DAYS IN 0 mg/l ABS WATER CULTURE

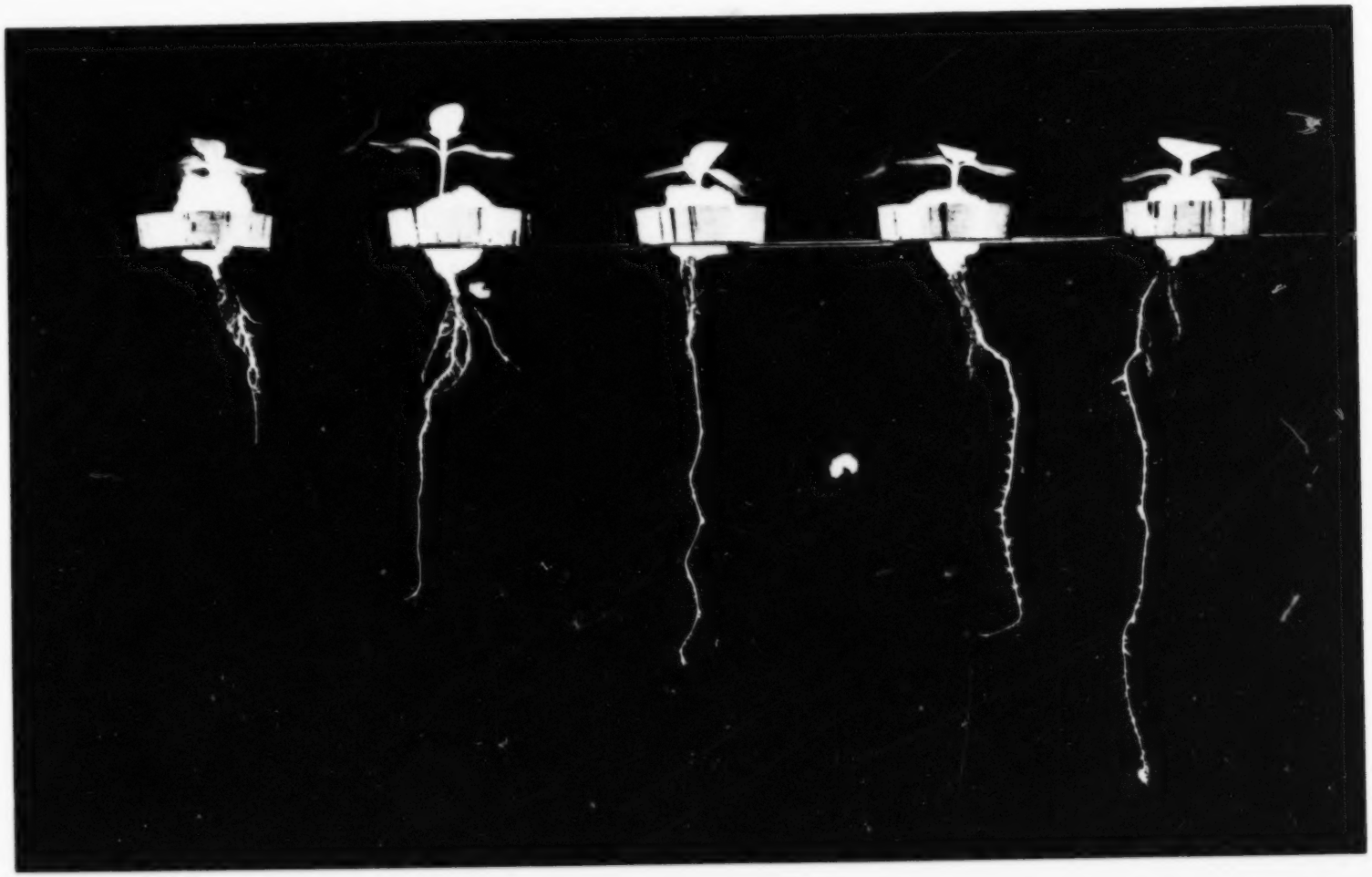

FIGURE 6. SUNFLOWERS GROWN 5 DAYS IN 10 mg/l ABS WATER CULTURE

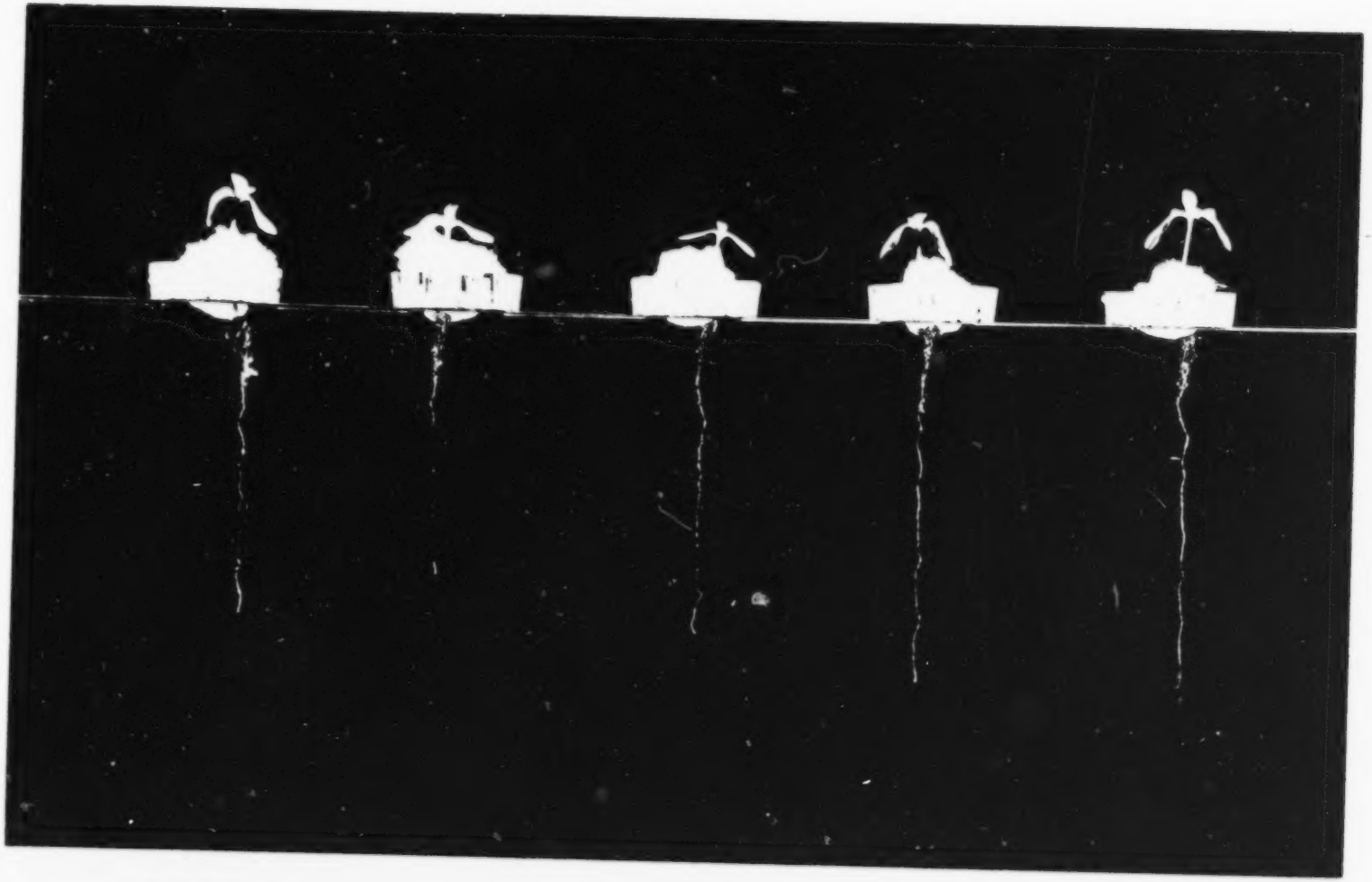

FIGURE 7. SUNFLOWERS GROWN 5 DAYS IN 40 mg/l ABS WATER CULTURE

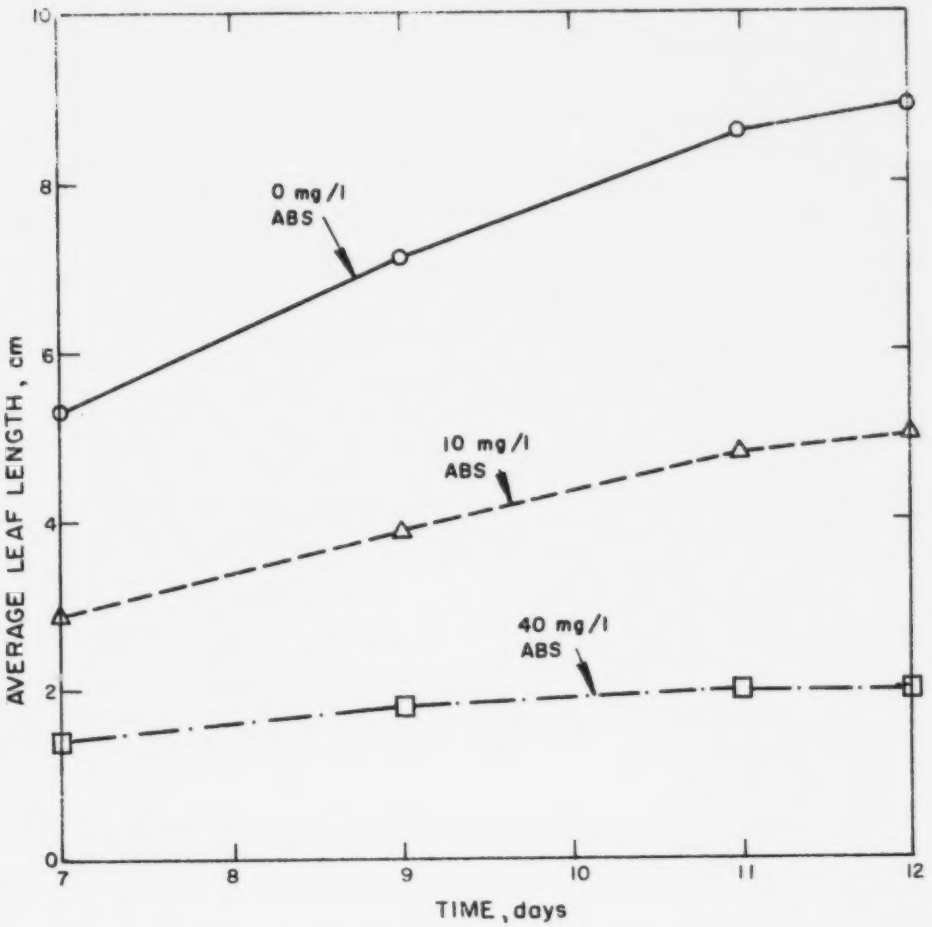

FIGURE 8. GROWTH OF SUNFLOWERS IN WATER CULTURE, LEAF MEASUREMENTS

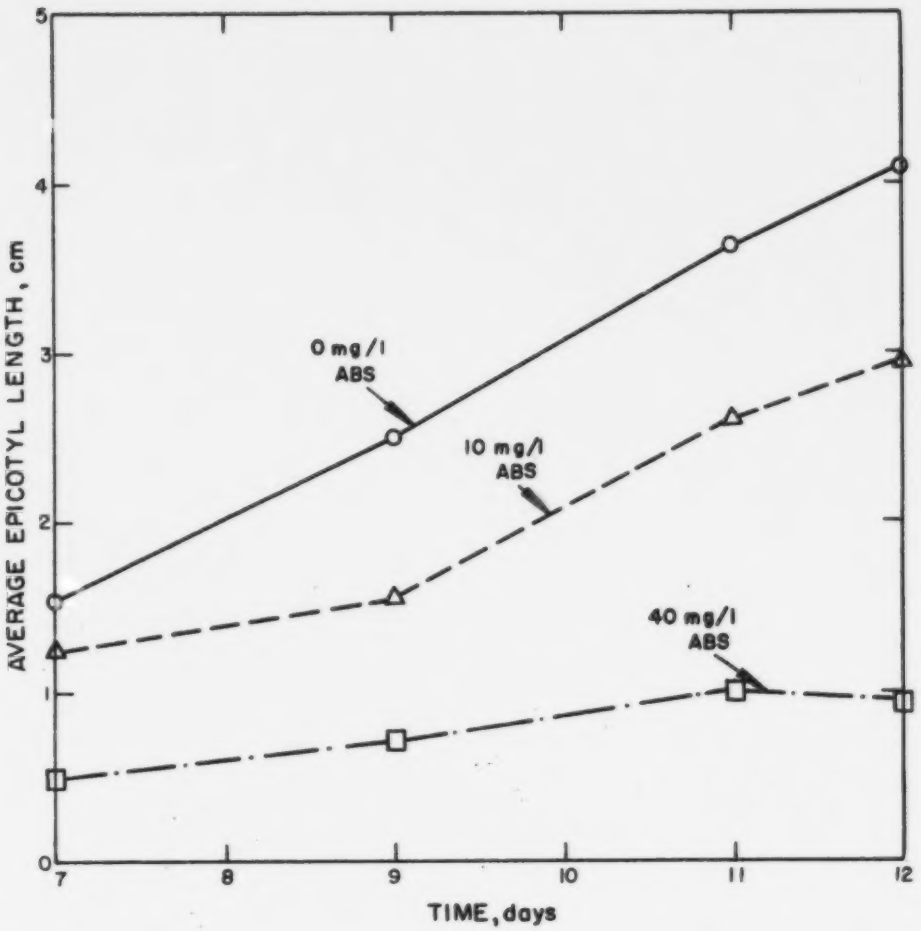

FIGURE 9. GROWTH OF SUNFLOWERS IN WATER CULTURE, EPICOTYL MEASUREMENTS

FIGURE 10. SUNFLOWERS GROWN 12 DAYS IN 0 mg/l ABS WATER CULTURE

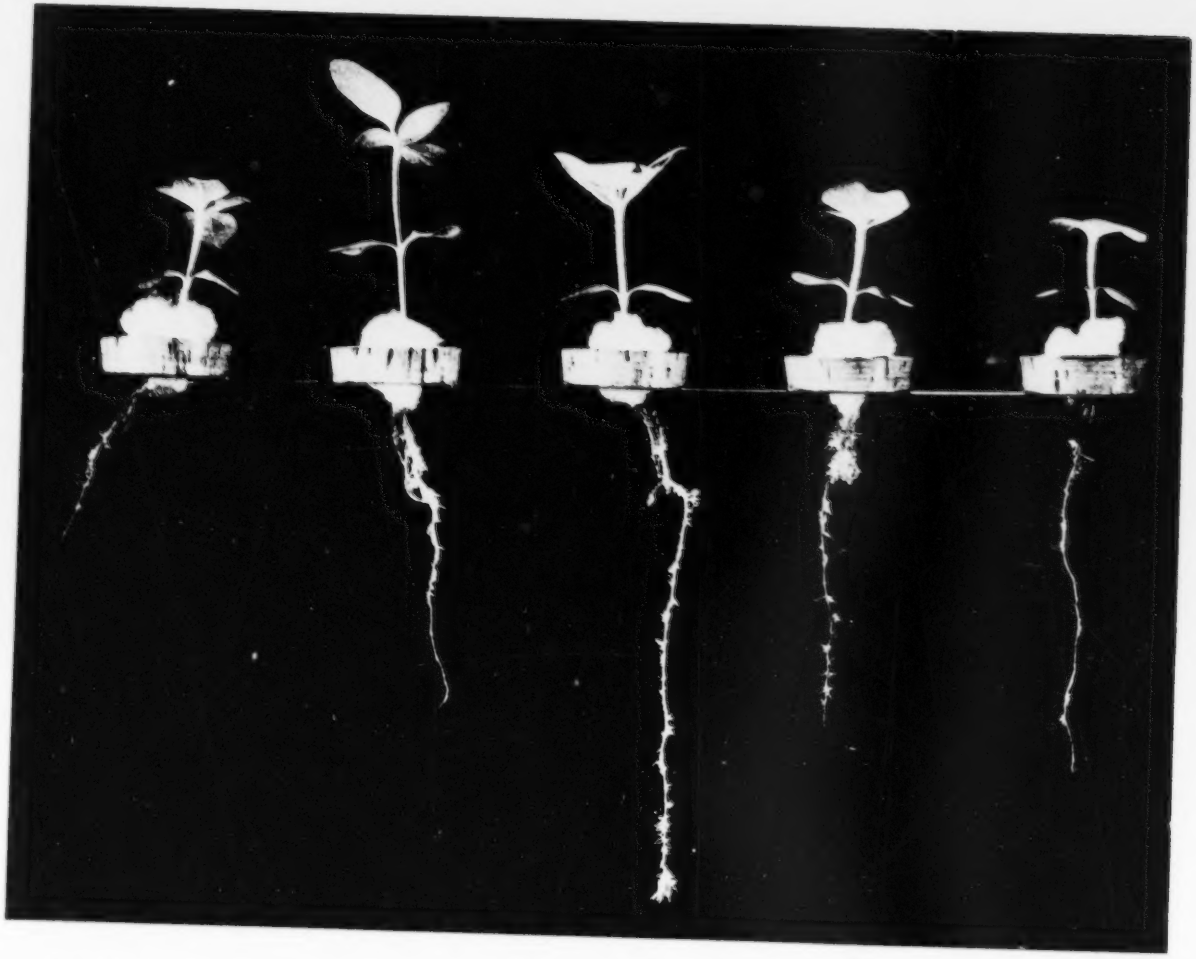

FIGURE 11. SUNFLOWERS GROWN 12 DAYS IN 10 mg/l ABS WATER CULTURE

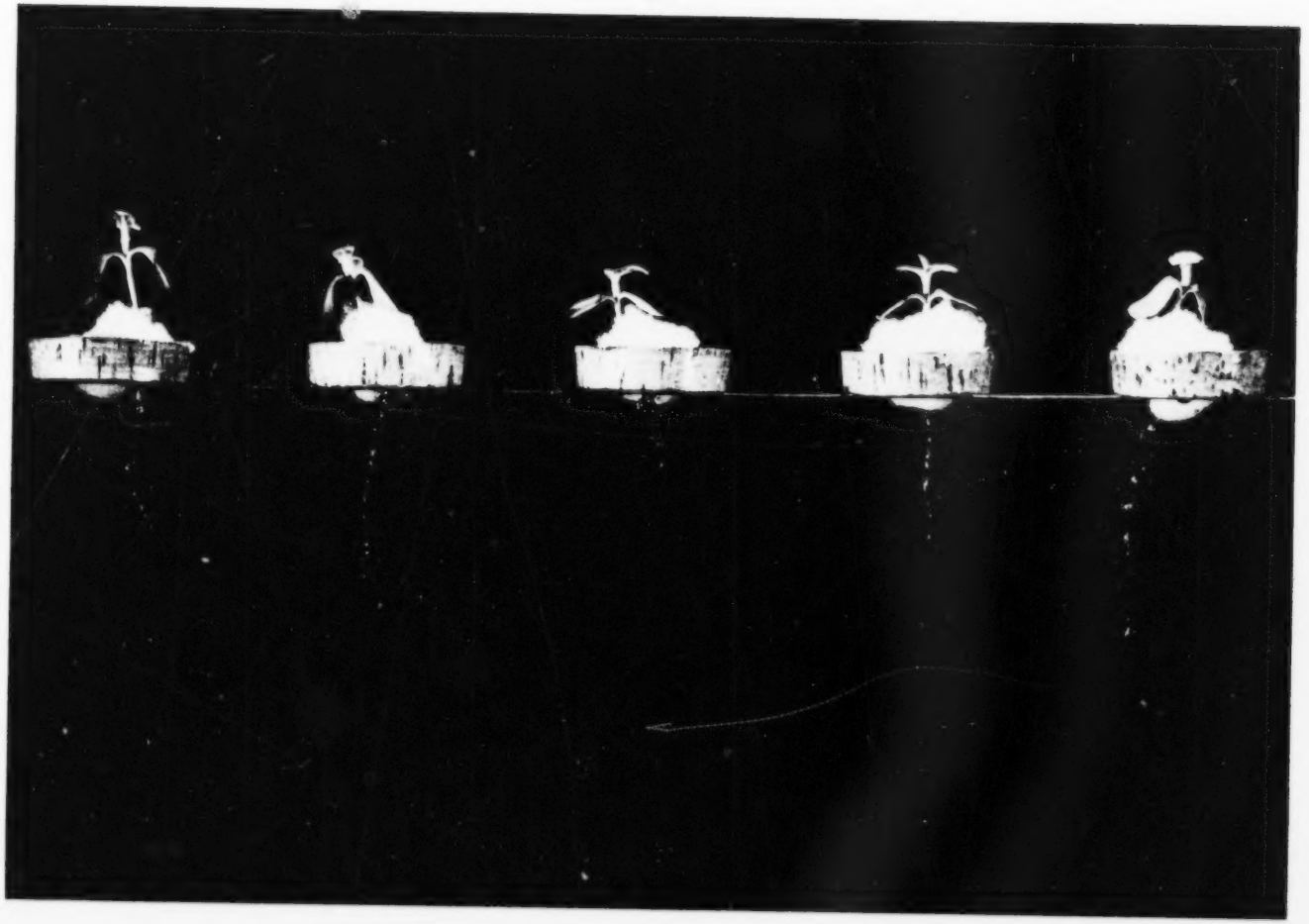

FIGURE 12. SUNFLOWERS GROWN 12 DAYS IN 40 mg/l ABS WATER CULTURE

FIGURE 13. CHLOROSIS OF LEAVES OF SUNFLOWERS GROWN 12 DAYS IN 10 mg/l ABS WATER CULTURE

TABLE I

FINAL WEIGHT OF SUNFLOWERS GROWN IN WATER CULTURE

ABS mg/l	Fresh Weights (g)						5-Plant Total Dry Wt. (g)		Water Content (%) Fresh Wt. Basis	
	Roots			Stems & Leaves						
	5-Plant Total	$\bar{X}$	s	5-Plant Total	$\bar{X}$	s	Roots	Stems & Leaves	Roots	Stems & Leaves
0	12.29	2.46	0.48	30.40	6.08	0.86	1.10	2.50	91.1	91.8
0	15.08	3.02	0.45	34.06	6.81	0.93	1.50	3.40	90.1	90.0
0	10.26	2.05	0.67	21.16	4.23	0.93	0.80	1.50	92.2	92.9
10	1.66	0.33	0.11	8.35	1.67	0.49	0.24	0.76	85.7	90.9
10	1.91	0.38	0.10	8.84	1.77	0.30	0.26	0.86	86.7	90.2
10	1.98	0.40	0.09	8.98	1.80	0.21	0.28	0.84	85.7	90.6
40	0.16	0.03	0.01	3.27	0.66	0.17	0.06	0.48	62.8	85.4
40	0.15	0.03	0.01	3.33	0.67	0.14	0.05	0.36	64.4	89.2
40	0.16	0.03	0.01	2.67	0.53	0.09	0.05	0.41	65.4	84.7
Average										
0	12.54	2.51	0.53	28.54	5.71	0.91	1.13	2.47	91.1	91.6
10	1.85	0.37	0.10	8.72	1.75	0.33	0.26	0.82	86.0	90.6
40	0.16	0.03	0.01	3.09	0.62	0.13	0.05	0.42	64.2	86.4

Note: Total weights represent the sum of five plants grown in each container, $\bar{X}$ is the mean weight, and s is the sample standard deviation.

TABLE II

UPTAKE OF ABS (ABS^{35}), S (S^{35}) BY SUNFLOWERS

Container Liquid ABS Conc. (mg/l)	Plant Material	Plant Uptake of S^{35}, S				Plant Uptake of ABS^{35}, ABS				ABS Degradation per Plant			
		As S^{35} cpm/5 Plants	As S			As ABS^{35} cpm/5 Plants	As ABS			Partial		Complete (to $BaS^{35}O_4$)	
			µg/5 Plants	µg/ Plant	µg/g Dry Wt.		µg/5 Plants	µg/ Plant	µg/g Dry Wt.	(µg)	(%)	(µg)	(%)
10	Leaves & Stems	3,850	132	26	174	2,180	75	15	98	11	43	.01	.04
	Roots	34,600	1,190	238	5,000	30,300	1,040	208	4,380	30	13	.01	.004
10	Leaves & Stems	3,900	134	27	159	2,700	93	19	110	8	30	.00	.00
	Roots	39,500	1,360	272	4,800	35,000	1,200	240	4,260	32	12	.01	.004
10	Leaves & Stems	3,200	111	22	128	1,320	45	9	53	13	59	.00	.00
	Roots	32,800	1,130	226	4,420	31,600	1,090	218	4,260	8	4	.02	.009
40	Leaves & Stems	7,180	1,150	230	3,180	5,640	902	180	2,500	50	22	.03	.01
	Roots[b]	32,000	5,120	1,024	93,100	32,000	5,120	1,024	93,100	0	0	.09	.009
40	Leaves & Stems	12,100	1,940	388	4,050	7,700	1,230	246	2,570	142	37	.06	.02
	Roots[b]	32,000	5,120	1,024	93,100	32,000	5,120	1,024	93,100	0	0	.09	.009
40	Leaves & Stems	13,100	2,100	420	5,140	5,250	840	168	2,060	252	60	.00	.00
	Roots[b]	32,000	5,120	1,024	93,100	32,000	5,120	1,024	93,100	0	0	.09	.009

[a]Partial degradation is the difference between total sulfur (S) per plant and ABS per plant.

[b]Plant roots grown in 40 mg/l ABS were combined into one sample due to insufficient material for individual analyses; figures shown in these columns represent one-third of the total sample.

TABLE III

AVERAGE UPTAKE OF ABS AND S BY SUNFLOWERS GROWN IN WATER CULTURE

Water Culture ABS (mg/l)	Plant Part	S (µg/g, Dry Weight)	ABS (µg/g, Dry Weight)	Degradation of ABS (%)
10	Stems & Leaves	154	87	43.5
10	Roots	4,740	4,300	9.3
40	Stems & Leaves	4,123	2,377	42.4
40	Roots	93,100	93,100	0.0

Degradation products of ABS were found predominantly in the stems and leaves, which would seem to indicate that this is where degradation occurs. However, it is possible that degradation actually occurs in the roots, and that these degradation products are then rapidly translocated to the stems and leaves. Table II shows that although sizeable quantities of ABS were partially degraded (an average of 42 per cent in the stems and leaves), almost no ABS-related SO_4 was found in plant parts. This is not surprising since plants readily utilize SO_4 in their metabolism, and it may therefore be assumed that significant amounts of ABS were completely degraded to SO_4 which was then incorporated into a complex form.

Barley Experiment. The experimental procedure for growing barley was similar to that for sunflowers. Atlas barley was germinated in vermiculite and then grown for 14 days in nutrient water culture containing ABS concentrations of 0, 10, and 40 mg/l. Tracer ABS^{35} was added not only to the test containers, but also to one of the controls. To eliminate the remote possibility of a synergistic effect with ABS on the test plants, no foam depressant was used.

The apparatus was altered slightly to give needed support to the barley seedlings. Individual supports were constructed using thread wound around two upright glass rods (cf. Figure 1).

Plant growth was noted during the course of the experiment by measurement of shoot length. Each new shoot was measured from the ligule of the old shoot.

Figure 14 shows the barley seedlings on the third day after their immersion in water culture. At this point there is no evidence of retardation of growth. This photograph may be contrasted with one taken after 14 days (Figure 15) demonstrating the serious toxicity of ABS which became apparent during the course of the experiment. Figure 16 depicts the average growth of the second shoots which is typical of over-all plant growth. Again, there was virtually no growth at 40 mg/l ABS, and

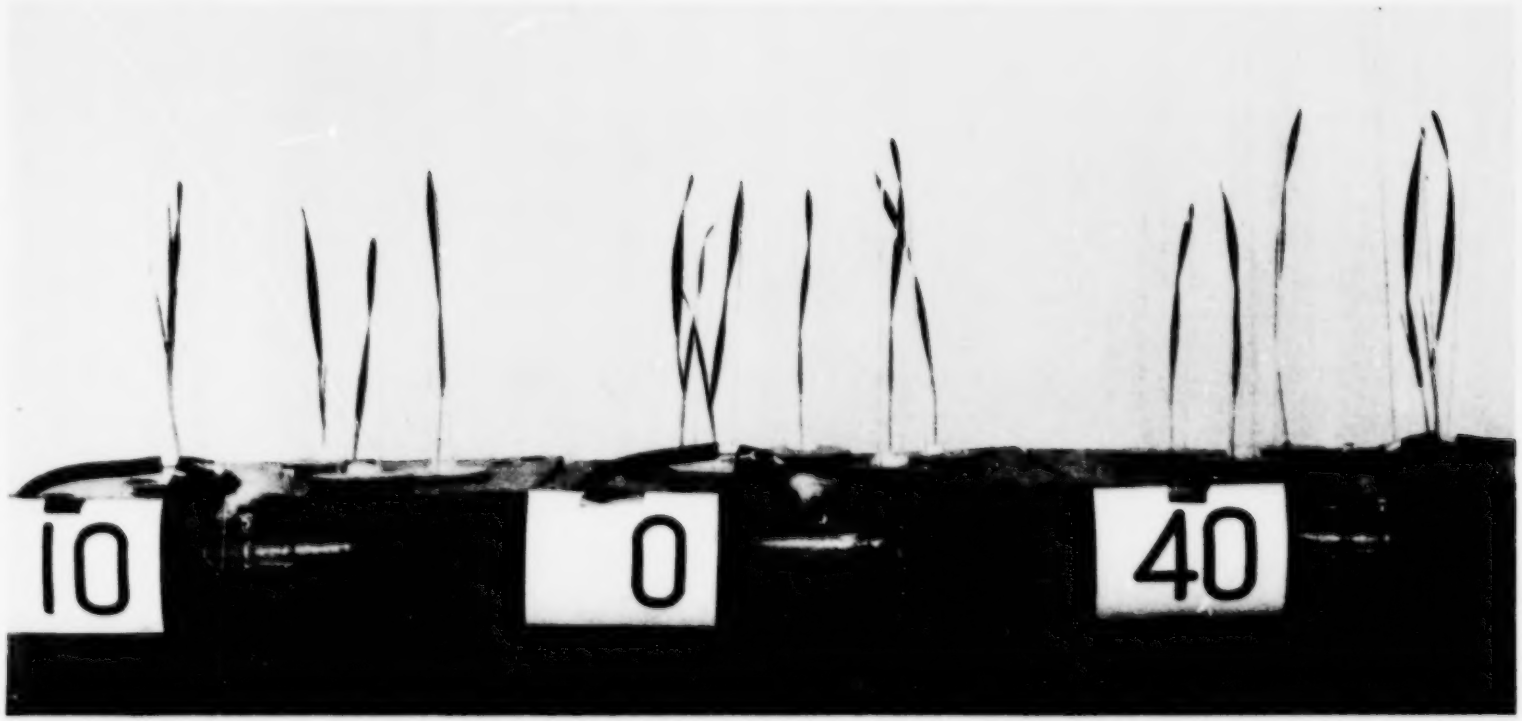

FIGURE 14. BARLEY GROWN 3 DAYS IN WATER CULTURE AT INDICATED ABS CONCENTRATIONS (mg/l)

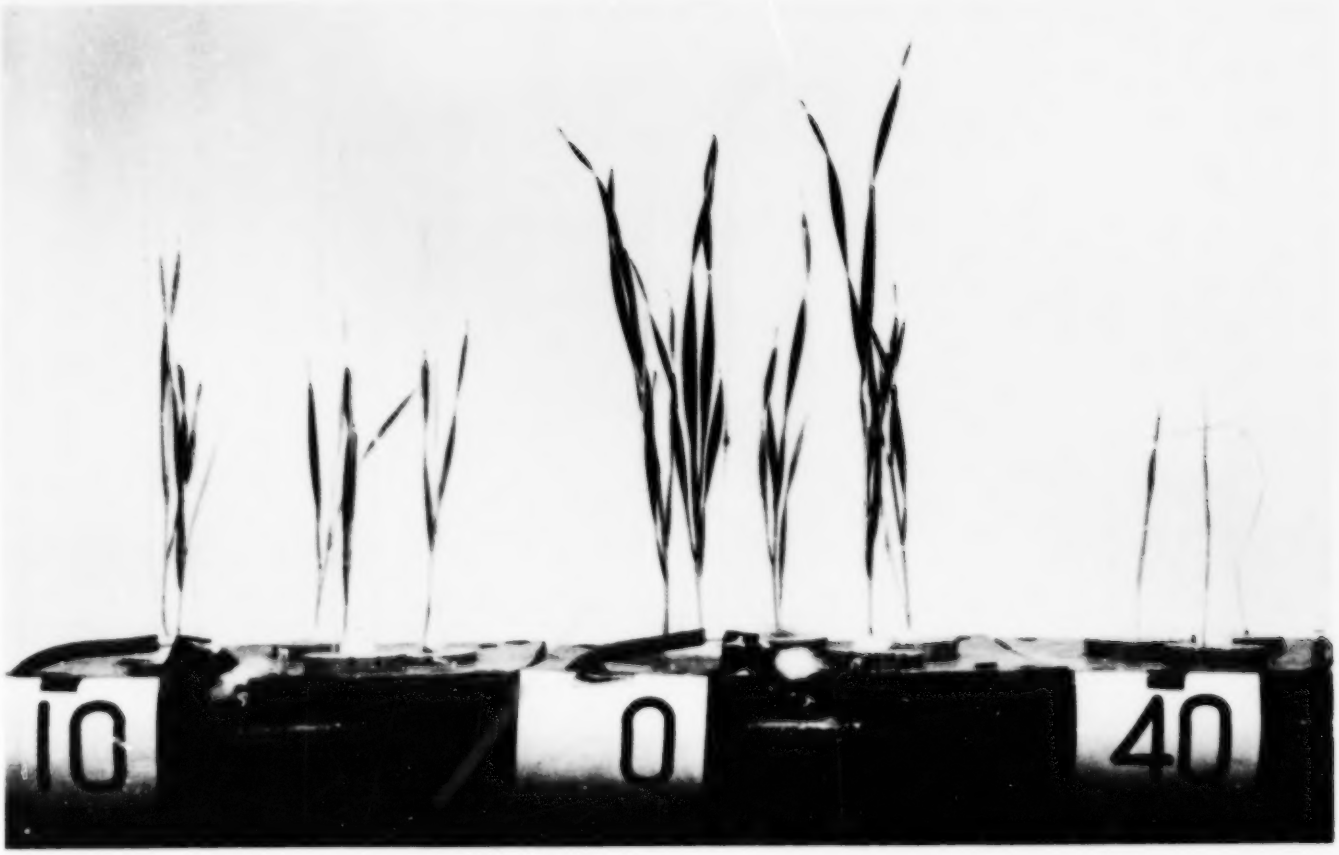

FIGURE 15. BARLEY GROWN 14 DAYS IN WATER CULTURE AT INDICATED ABS CONCENTRATIONS (mg/l)

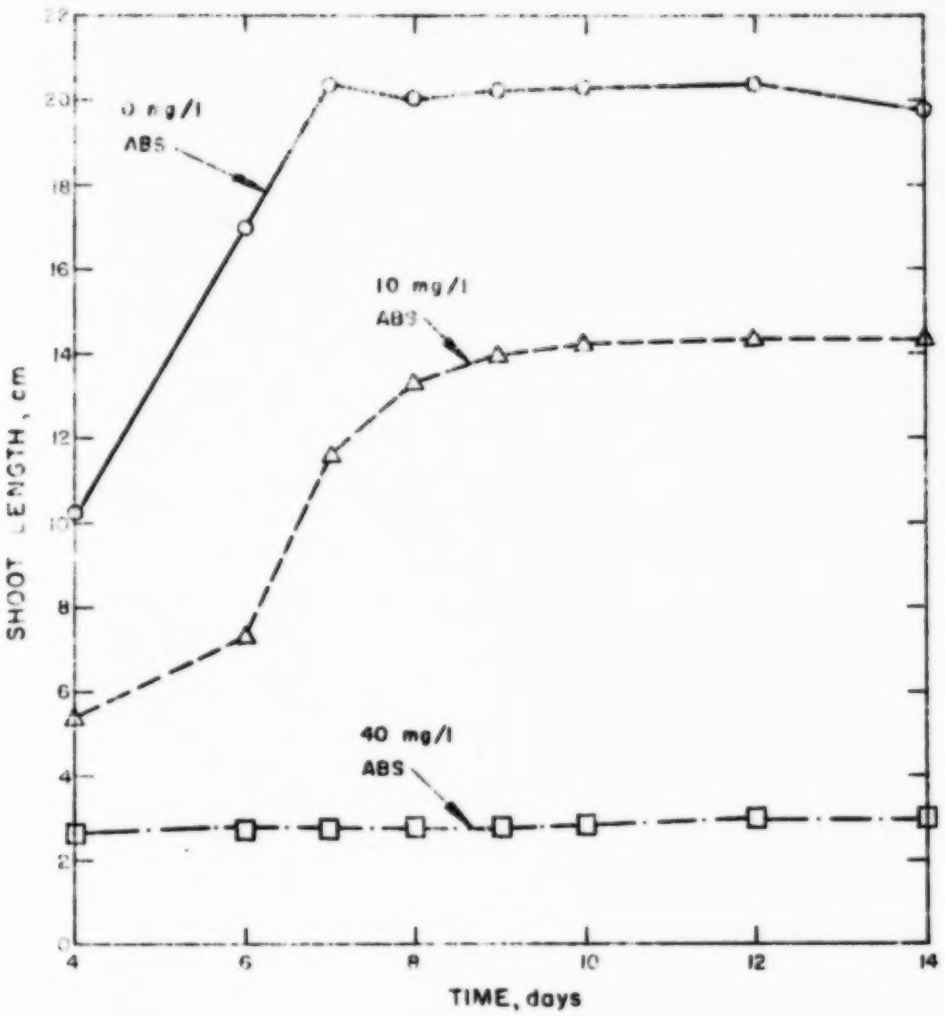

FIGURE 16. GROWTH OF BARLEY (2nd Shoot) IN WATER CULTURE

as shown in Table IV the mean fresh weight of the control roots was more than twice the 10 mg/l group and approximately 6 times the 40 mg/l group. A similar comparison of mean shoot weight would be 3 and 8 times, respectively. The water content of plants was severely reduced as ABS concentration increased.

TABLE IV

EFFECT OF ABS CONCENTRATION ON BARLEY GROWTH

ABS (mg/l)	Fresh Weights (g)				5-Plant Total Dry Wt. (g)		Water Content (%) Fresh Wt. Basis	
	Roots		Shoots					
	5-Plant Total	$\bar{X}$	5-Plant Total	$\bar{X}$	Roots	Shoots	Roots	Shoots
0	1.62	0.32	5.69	1.14	0.25	0.58	84.5	89.7
0	1.67	0.33	6.11	1.22	0.30	0.67	82.1	89.0
0	1.75	0.35	5.44	1.09	0.21	0.55	88.3	89.8
10	0.99	0.20	1.88	0.38	0.19	0.28	80.7	85.2
10	0.61	0.12	1.71	0.34	0.18	0.27	70.6	84.0
10	0.61	0.12	1.82	0.36	0.20	0.26	67.9	85.8
40	0.32	0.06	0.82	0.16	0.10	0.20	67.8	75.9
40	0.28	0.06	0.85	0.17	0.10	0.20	63.6	76.2
40	0.29	0.06	0.57	0.11	0.12	0.18	59.3	68.2
Average								
0	1.68	0.34	5.75	1.15	0.25	0.60	85.0	89.5
10	0.74	0.15	1.80	0.36	0.19	0.27	73.1	85.0
40	0.29	0.06	0.75	0.15	0.11	0.19	63.6	73.4

Note: Total weight represents the sum of five plants grown in each container; $\bar{X}$ represents the mean weight.

Results of radioassay, reported fully in Table V and summarized in Table VI, again showed that there was a predominance of ABS in the roots and of ABS degradation products in the shoots. Unlike the sunflower experiment, however, barley's uptake of ABS increased almost in proportion to the ABS concentration in the water culture. A 4-fold increase in water culture ABS concentration resulted in a 5-fold increase in uptake in the roots and a 6.7-fold increase in the shoot uptake of ABS, as opposed to the sunflower experiment where more than a 20-fold increased uptake occurred under both circumstances.

TABLE V

UPTAKE OF ABS (ABS^{35}) AND S (S^{35}) BY BARLEY

Container Liquid ABS Conc. (mg/l)	Plant Material	Plant Uptake of S^{35}, S				Plant Uptake of ABS^{35}, ABS				ABS Degradation per Plant			
		As S^{35} cpm/ 5 Plants	As S			As ABS^{35} cpm/ 5 Plants	As ABS			Partial[a]		Complete (to $BaS^{35}O_4$)	
			µg/5 Plants	µg/ Plant	µg/g Dry Wt.		µg/5 Plants	µg/ Plant	µg/g Dry Wt.	(µg)	(%)	(µg)	(%)
10	Shoots	7,700	82	16	320	3,600	37	9	143	7	44	0.00	0.00
	Roots	87,300	930	186	4,700	86,500	890	178	4,520	8	4	.01	.005
10	Shoots	7,500	80	16	290	4,100	42	8	151	8	50	.00	.00
	Roots	86,300	920	184	4,800	66,800	690	138	3,590	46	25	.01	.005
10	Shoots	8,100	86	17	310	3,100	32	6	117	11	65	.00	.00
	Roots	83,700	890	178	5,000	72,500	740	148	4,160	30	17	.01	.006
40	Shoots	8,200	380	76	1,580	3,700	157	31	800	45	59	.00	.00
	Roots[b]	44,300	2,100	420	20,400	46,700	2,080	416	20,200	4	1	.03	.007
40	Shoots	10,200	470	94	1,970	5,200	231	46	1,150	48	51	.00	.00
	Roots[b]	44,300	2,100	420	21,000	46,700	2,080	416	20,800	4	1	.03	.007
40	Shoots	6,700	310	62	980	3,700	157	31	860	31	50	.00	.00
	Roots[b]	44,300	2,100	420	18,100	46,700	2,080	416	18,000	4	1	.03	.007

[a]Partial degradation is the difference between total sulfur (S) per plant and ABS per plant.

[b]Plant roots grown in 40 mg/l ABS combined into one sample due to insufficient material for individual analyses; figures shown in these columns represent one-third of the total sample.

TABLE VI

AVERAGE UPTAKE OF ABS AND S BY BARLEY GROWN IN WATER CULTURE

Water Culture ABS (mg/l)	Plant Part	S (μg/g, Dry Weight)	ABS (μg/g, Dry Weight)	Degradation of ABS (%)
10	Shoots	310	140	55.0
10	Roots	4,800	4,100	14.6
40	Shoots	1,500	940	37.3
40	Roots	20,000	20,000	00.0

Considering the average uptake of ABS by the barley plants, approximately 8 μg were present in the shoot and 155 μg in the root at 10 mg/l. At 40 mg/l, there were 36 μg in the shoot and 416 μg in the root. From these results it can be seen that under comparable conditions individual barley plants take up on the order of 50 per cent less ABS than do sunflowers. Therefore, the ability of plants to concentrate ABS, and thereby aid in prevention of ground water contamination by ABS, varies considerably with different species.

Plant Growth in Soil

Sunflower Experiment. Sunflower plants germinated in vermiculite were grown in Yolo sandy loam contained in 7-inch flower pots. Drainage was provided by an underdrain of Fairoaks pea gravel overlaid with Monterey coarse and Monterey fine sand. Five solutions consisting of tap water, settled sewage, and tap water containing 5, 10, and 50 mg/l ABS were each applied to five replicate pots for a period of 57 days. These solutions all contained tracer ABS^{35} and were administered in 1-liter quantities twice weekly. One liter was sufficient to exceed soil saturation capacity and flush out excess salts, thereby preventing undesirable salt buildup in the soil. No fertilizer was added to the soil, nor were any nutrients added to the solutions. At the conclusion of the experiment soil analyses were made for adsorbed and degraded ABS^{35}.

The growth curves shown in Figure 17 demonstrate that under the experimental conditions the plants fed sewage effluent flourished compared with those receiving tap water. This demonstrates that the beneficial effects of nutrients outweigh the harmful effects of ABS in sewage for plants grown in nutrient-deficiient soil. As found in the water culture studies, plants grown at 10 and 50 mg/l ABS were retarded. Figure 18 illustrates pictorially a typical plant grown at each ABS concentration from which it is evident that retardation occurring at 10 and 50 mg/l ABS is not as severe in soil as in water culture.

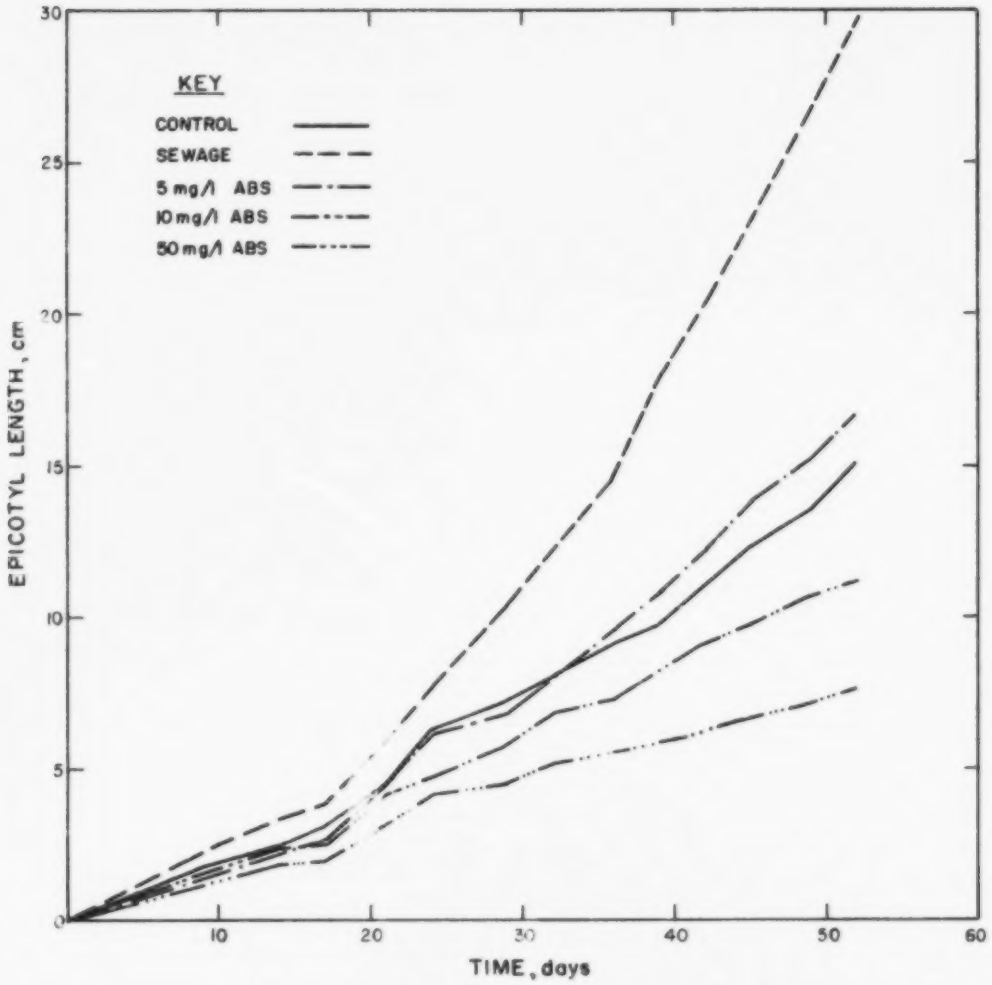

FIGURE 17. GROWTH OF SUNFLOWERS IN SOIL

FIGURE 18. SUNFLOWERS GROWN IN UNFERTILIZED SOIL (PLANT GROWN IN SEWAGE IS CONTRASTED WITH THOSE IRRIGATED WITH VARIOUS ABS CONCENTRATIONS, mg/l, IN TAP WATER)

Although the 5-mg/l plants grew slightly taller than the controls, Table VII shows that the control plants averaged slightly more fresh weight. However, neither of these differences was statistically significant. The mean fresh weight of the controls was approximately 3 times the 50-mg/l plants, 1.4 times the 10-mg/l plants, the same as the 5 mg/l plants, but 7 times less than the sewage-fed plants.

TABLE VII

EFFECT OF ABS ON SUNFLOWERS GROWN IN SOIL

ABS in Water (mg/l)	Mean Fresh Wt. (g)	Mean Dry Wt. (g)	Mean Water Content (Fresh Wt. Basis) (%)
0	3.72	0.80	78.5
5	3.62	0.75	79.2
10	2.61	0.58	77.5
50	1.23	0.33	73.0
Sewage[a]	26.96	5.29	80.5

[a]ABS concentration in sewage ranged from 4.6 to 12.7 mg/l.

Plant analyses reported in Table VIII show less ABS uptake in the stems and leaves than when plants were grown in water culture (51.5 per cent less at 10 mg/l and 96.1 per cent less at 50 mg/l). In both soil and water cultures, at 10 mg/l ABS the uptake by the plants of S related to ABS was approximately the same, but breakdown of ABS was almost double in the plants grown in soil. This suggests that the extended growing period allows more time for breakdown of ABS to occur both in soil and in the plant. Another factor appears to be a difference in the mechanisms of ABS uptake by plants grown in the two media. At 50 mg/l ABS, plants grown in soil contained only 39.3 per cent as much ABS as the plants grown at 40 mg/l in water culture, and had but 17.4 per cent ABS related S as the latter. From these results it is evident that ABS toxicity studies in water culture greatly exaggerate adverse effects on plant growth.

Despite the decrease in plant ABS uptake noted in soil studies, this uptake is still sizeable enough to be of significance in reducing the ABS content of soils in the root zone. For example, the sewage-fed plants took up an average of 247.0 µg of ABS-related S per gram dry weight of plant material (stems and leaves). The average dry weight of these plants was 5.29 g, giving a total uptake in the stems and leaves of 1,304 µg. This is 3.3 per cent of the ABS adsorbed on the soil in the pots. An estimate of root ABS uptake at double this figure (water culture studies showed a root-to-stem ABS concentration ratio of

TABLE VIII

UPTAKE OF ABS AND S BY SUNFLOWERS GROWN IN SOIL

ABS in Water (mg/l)	Average Plant S (μg/g Dry Wt.)	Average Plant ABS (μg/g Dry Wt.)	Average Degraded ABS in Plant (%)
5	165.0	34.2	79.4
10	186.0	43.0	77.0
50	719.0	93.5	87.0
Sewage[a]	247.0	79.2	67.9

[a]ABS concentration in sewage ranged from 4.6 to 12.7 mg/l.

approximately 10-to-1 for 10-mg/l sunflowers, and a weight ratio of approximately 1-to-5) would mean that these plants might remove ten per cent of the ABS adsorbed on the soil in the root zone.

Soil analyses reported in Table IX show a large sample standard deviation (s) within each treatment group. Nevertheless, there is a rough parallel between liquid ABS concentration and average soil ABS adsorption. No ABS-related SO_4 was present in any sample, but in view of plant uptake and metabolism of SO_4, the $BaSO_4$ test for ABS degradation is inconclusive for this study. However, an estimate of ABS degradation may be made from the expected ABS adsorption based on previous Yolo sandy loam column studies at a comparable loading. The calculated loading per pot in the present study was 1.37 mgd/acre-ft. In a time experiment conducted on columns of Yolo sandy loam, one column, infiltrated with a 1-mg/l ABS solution for one hour, had a comparable loading of 1.25 mil gal/acre-foot with a resultant ABS adsorption of 1.54 μg/g. For a 5-mg/l ABS infiltrate the expected adsorption would be 7.7 μg/g, since the isotherm is linear with ABS concentration. Average adsorption at 5 mg/l was actually 7.3 μg/g, indicating that little degradation occurred. Similarly, in the pots receiving 10 and 50 mg/l ABS only 20 per cent of the calculated amount of adsorbed ABS was degraded during the course of the experiment (including plant uptake of ABS in the stems and leaves which was negligible in this calculation; plant roots were not analyzed). Neither degradation nor plant uptake appear to be of sufficient magnitude to reduce the ABS content of soils enough to prevent ground-water contamination during intermittent water irrigation over a considerable period of time.

Barley Experiment. The results of the sunflower experiment indicated a need for separation of the two critical experimental factors in plant growth--nutrients and ABS. This was accomplished in an experiment with barley as the test plant by applying water solutions of ABS to one group of plants, and settled sewage plus ABS to another group. From

TABLE IX

FINAL SOIL ABS CONCENTRATION IN μg/g
(Sunflower Experiment)

Treatment - ABS Concentration in Water (mg/l)			
5	10	50	Sewage[a]
4.6	16.6	65.7	17.7
4.4	15.6	91.0	9.0
11.0	4.8	53.1	11.5
6.7	15.4	36.8	15.1
9.5	9.5	58.0	15.2
Average			
7.3	12.4	61.0	17.7
s			
2.9	5.1	19.8	3.4

[a]ABS concentration in sewage ranged from 4.6 to 12.7 mg/l.

Note: The $BaSO_4$ test for ABS-related degradation to SO_4 was negative for all samples.

the sunflower experiment it was also evident that the soil, Yolo sandy loam, was deficient in nutrients. Chemical fertilizers were added (cf. Appendix A) to 1600 g of soil in each pot. Nine barley seeds were planted per pot and six days later the four smallest seedlings were removed. The first group of 25 pots was divided into replicate groups of five and watered twice a week with distilled water containing 0, 5, 10, 15, and 20 mg/l ABS. A second group of 20 pots, also divided into replicate groups of five, received settled sewage containing added ABS in concentrations of 0, 5, 10, and 15 mg/l. There were thus 25 plants tested at each treatment, and tracer ABS^{35} was present in all solutions. Plants were grown in direct sunlight for a period of 57 days.

The group receiving distilled water solution of ABS (0.7 liter per watering) was provided with saucers to retain excess water and prevent loss of nutrients by leaching. The settled sewage group (1 liter per watering) was allowed to drain freely to avoid salt buildup. Loss of nutrients was no problem, since they were replenished by the sewage.

Plant height, as measured from soil to tallest leaf, was logged once a week. Figure 19 shows the average heights of the 25 plants at

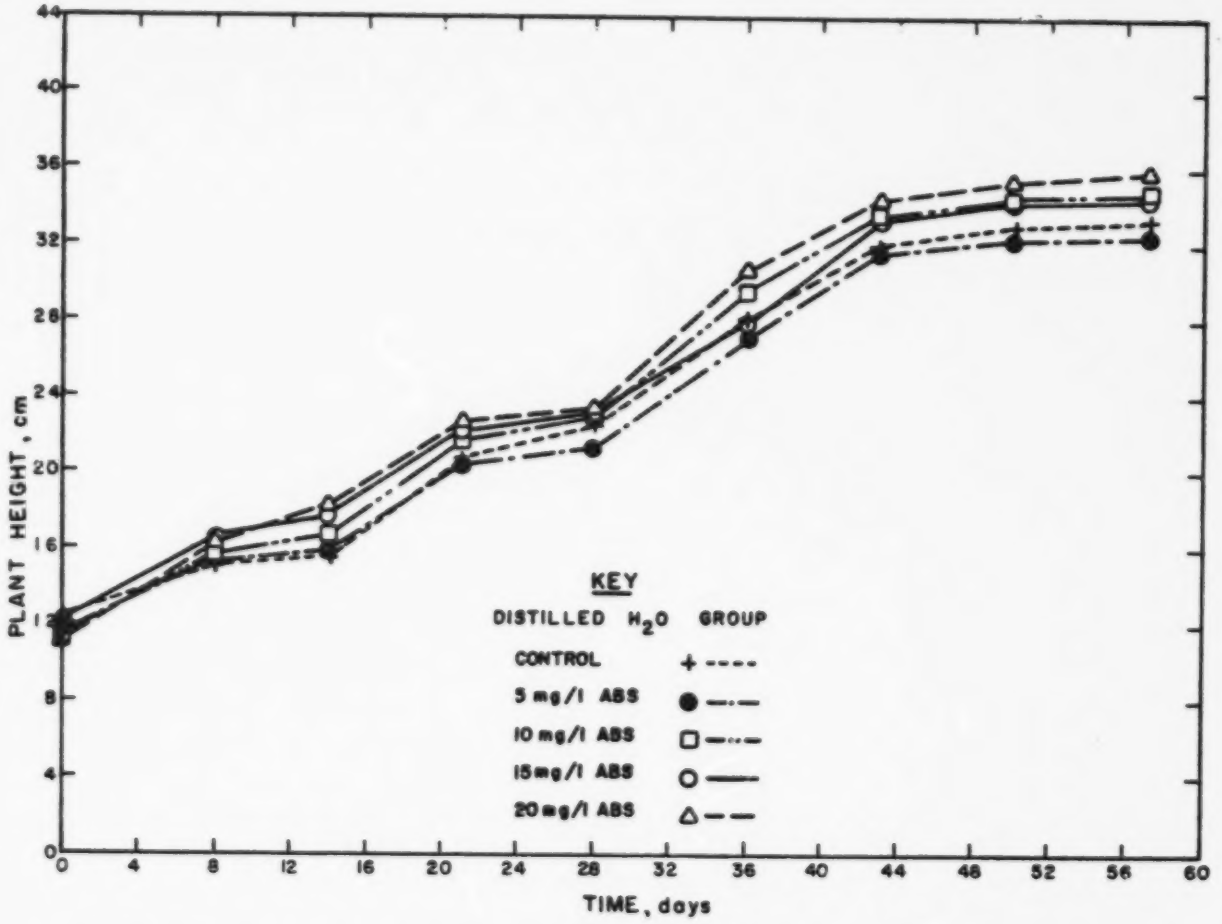

FIGURE 19. AVERAGE PLANT HEIGHT OF BARLEY GROWN IN FERTILIZED SOIL WITH DISTILLED WATER – ABS SOLUTIONS

each ABS concentration in distilled water, and Figure 20 depicts the same parameter for the plants receiving settled sewage containing ABS. There was no significant difference between the control and test plants within each group. However, comparing the two groups, the plants receiving settled sewage were a statistically significant 1.2 times taller.

Other parameters of growth, summarized in Table X, are number of leaves, number of awns, fresh weight, and grain weight. No significant within-group differences were found for any of these parameters, but between-group differences were several-fold. Plants irrigated with settled sewage were 2.7 times heavier, yielded 2.3 times more grain, had 4.2 times more awns and 4.4 times more leaves. Figures 21 and 22 give pictorial evidence of the growth difference between the two groups. It is evident that barley, grown in fertilized soil, is insensitive to ABS but responsive to nutrients in sewage over and above normal fertilization practices.

TABLE X

BARLEY GROWTH IN SOIL

Treatment	Fresh Weight (g)	Grain Weight (g)	Number of Awns	Number of Leaves
Distilled Water	11.5	2.5	25	144
Distilled Water + 5 mg/l ABS	10.7	2.2	26	128
Distilled Water + 10 mg/l ABS	12.4	2.8	26	154
Distilled Water + 15 mg/l ABS	11.6	2.4	24	130
Distilled Water + 20 mg/l ABS	13.2	2.3	30	127
Group Average	11.9	2.4	26.2	136.6
Settled Sewage	33.4	5.8	104	595
Settled Sewage + 5 mg/l ABS	30.5	4.9	118	620
Settled Sewage + 10 mg/l ABS	35.8	6.4	119	656
Settled Sewage + 15 mg/l ABS	31.1	5.0	98	529
Group Average	32.7	5.5	109.8	600

Note: Figures shown denote the final 25-plant average at each ABS concentration.

After 57 days, irrigation was suspended to harden the barley grain. Two weeks later the grain was harvested and analyzed for ABS uptake. It was dried to a constant weight and milled to a fine powder. The initial total activity (S^{35}) count was made on the powder which was then double extracted with 70 per cent ethanol, and then selectively ether-extracted for ABS^{35} analysis. $BaS^{35}O_4$ tests were carried out on the water phase for complete degradation to $S^{35}O_4$.

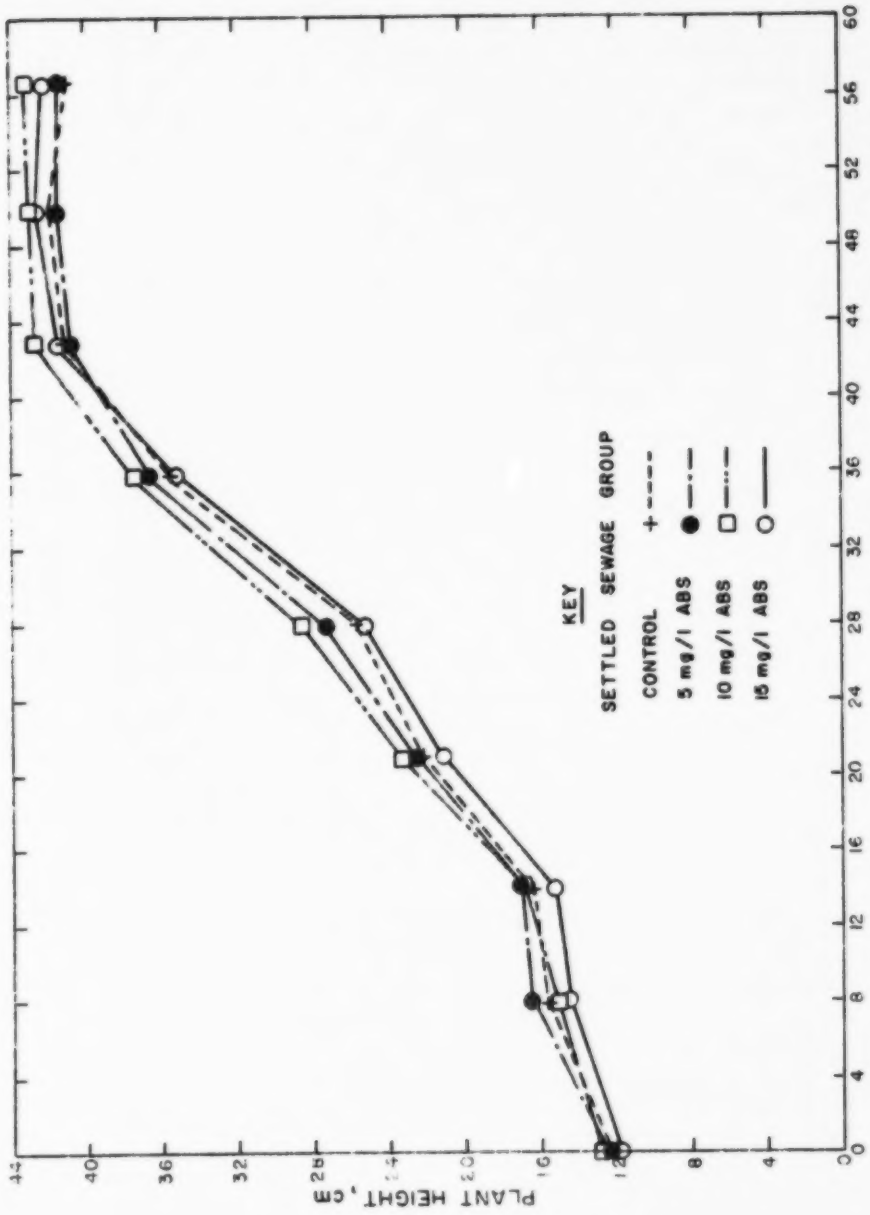

FIGURE 20. AVERAGE PLANT HEIGHT OF BARLEY GROWN IN FERTILIZED SOIL WITH SETTLED SEWAGE–ABS SOLUTIONS

FIGURE 21. BARLEY IRRIGATED WITH SETTLED SEWAGE-ABS SOLUTIONS AND GROWN IN FERTILIZED SOIL

FIGURE 22. BARLEY IRRIGATED WITH DISTILLED WATER-ABS SOLUTIONS AND GROWN IN FERTILIZED SOIL

Each result tabulated in Table XI represents the average of 25 plants. The maximum ABS concentration was only 6.8 μg/g and three samples had no ABS. An average of 97.1 per cent of the radiosulfur found in the grain was related to breakdown products of ABS, and an average of 2.1 per cent was degraded to sulfate. This gives a net of 95 per cent related to intermediate breakdown products. With only traces of unaltered ABS present in this typical monocotyledon, it may be assumed that irrigating other members of the same plant family with ABS-bearing liquid would not lead to a significant ABS content in their edible portion.

TABLE XI

AVERAGE CONCENTRATION OF ABS AND S IN BARLEY GRAIN
(Barley Grown in Soil)

Treatment	S (μg/g Dry Wt.)	ABS (μg/g Dry Wt.)	Degradation of ABS (%)	Degradation of ABS to SO_4 (%)
Distilled Water + 5 mg/l ABS	59	0.0	100	1.9
Distilled Water + 10 mg/l ABS	41	0.6	98.5	2.0
Distilled Water + 15 mg/l ABS	115	---[a]	---	2.8
Distilled Water + 20 mg/l ABS	92	6.8	92.8	4.3
Settled Sewage[b] + 0 mg/l ABS	37	4.0	89.0	2.4
Settled Sewage + 5 mg/l ABS	67	0.0	100	2.1
Settled Sewage + 10 mg/l ABS	72	0.6	99.2	1.2
Settled Sewage + 15 mg/l ABS	94	0.0	100	0.0

[a]Sample lost.

[b]Average concentration of ABS in settled sewage was 6.4 mg/l.

Lupinus albus Experiment. A third study in soils was made with Lupinus albus. The procedure was similar to the barley experiment except that all plants were irrigated with distilled water and no tracer was added. ABS concentrations of 0, 5, 10, 15, and 20 mg/l were each added to five replicate 7-inch pots containing one plant each. Two kilograms of Yolo sandy loam and sufficient chemical fertilizers to give the same concentration as used previously were added to each pot. Seeds were germinated in vermiculite and the seedlings transplanted to the pots, which were randomized by the "magic square" method. For 12 days seedlings were given distilled water. After initiation of ABS into the feed solution, plants were grown in artificial light at room temperature for 29 days.

During the course of the experiment petioles were counted weekly and their length recorded (cf. Table XII). Results give no indication of ABS toxicity to Lupinus albus.

TABLE XII

EFFECT OF ABS ON GROWTH OF *Lupinus albus* IN SOIL

Day	ABS in Distilled Water (mg/l)	Number of Petioles	Total Length of Petioles (cm)	Average Length of Petioles (cm)
1	0	18	88.0	4.9
	5	19	72.0	3.8
	10	18	63.5	4.5
	15	16	55.0	3.4
	20	20	80.0	4.0
7	0	28	176.7	6.3
	5	31	167.5	5.4
	10	29	151.8	5.2
	15	28	129.4	4.6
	20	31	166.6	5.4
14	0	39	261.4	6.7
	5	41	249.5	6.1
	10	37	212.0	5.7
	15	34	189.8	5.6
	20	39	229.3	5.9
21	0	45	333.4	7.4
	5	45	319.0	7.1
	10	41	260.7	6.4
	15	40	247.4	6.2
	20	46	309.3	6.7
29	0	44	346.3	7.9
	5	46	356.6	7.8
	10	42	299.7	7.1
	15	42	293.6	7.0
	20	49	343.9	7.0

Effect of ABS on Germinating Seeds

Lupinus albus Experiment. In previous experiments seedlings have been allowed to grow for at least one week before application of ABS commenced. Therefore, an investigation was hereby made to determine whether significant ABS effects are noticeable at the germination stage, and whether ABS, like many toxic substances, has a stimulatory effect at concentrations below its toxic threshold. Studies were carried out using germinating seeds of Lupinus albus. The use of these seeds for studying the effects of pharmacological products has been recommended by F. Murayama [4], and the procedure which she developed (described previously) was used in the present investigation.

The batches of Lupinus albus seedlings were incubated for 24 hours at 20°C in a water-saturated atmosphere. After incubation the hypocotyl lengths were measured and the percentage increase calculated for each seedling. Three groups of 35 seedlings were grown in nutrient solution containing 0, 2, and 5 mg/l ABS.

Table XIII shows the per cent increase in hypocotyl length and indicates the stimulatory effect of ABS. At 0 mg/l ABS there was a 99.3 per cent increase; at 2 mg/l ABS, a 101.6 per cent increase; and at 5 mg/l ABS, a 112.1 per cent increase. The mean percentage growth at 2 and 5 mg/l ABS was compared statistically with the growth at 0 mg/l ABS [5]. There was no significant difference between the 0 and 2 mg/l growth average at the 95 per cent confidence level, but the seedlings at 5 mg/l ABS showed a highly significant (99.75 per cent confidence level) growth increase over the controls.

A second run was made on Lupinus albus seeds with concentrations of 0, 5, 10, and 20 mg/l ABS in the test solutions, and the results are reported in Table XIV. Growth was again stimulated at 5 mg/l ABS, as well as at 10 and 20 mg/l ABS. Moreover, the greatest stimulation occurred at 20 mg/l. The statistical F test [6] showed a significant difference in mean hypocotyl lengths at the 99.5 per cent confidence level.

It must be concluded that ABS in concentrations from 5 to 20 mg/l has an increasingly stimulating effect on this stage of the growth of Lupinus albus.

Corn Experiment. Corn seeds were germinated at higher ABS concentrations than Lupinus albus and different techniques were used. Six beakers of one-liter capacity were lined with paper towels and filled with vermiculite. Nine seeds were placed between the towel liner and the glass of each beaker. They were positioned at a depth of two inches and spaced at equal intervals on the periphery of the liner. ABS concentrations of 0, 25, 50, 100, 200, and 500 mg/l were tested. Initially 350 ml of solution was added to the beakers, followed by weekly additions of 100 ml during the 21-day duration of the experiment.

The results (cf. Table XV) show essentially no effect between the control and the 25 and 50 mg/l ABS concentrations. However, at

TABLE XIII

EFFECT OF ABS ON GERMINATION OF *Lupinus albus* SEEDS

0 mg/l ABS				2 mg/l ABS				5 mg/l ABS			
Length of Hypocotyl (mm)			Growth Increase (%)	Length of Hypocotyl (mm)			Growth Increase (%)	Length of Hypocotyl (mm)			Growth Increase (%)
Initial	Final	Growth		Initial	Final	Growth		Initial	Final	Growth	
27	55	28	103.7	24	54	30	125.0	31	61	30	96.8
27	50	23	85.2	26	55	29	111.5	24	53	29	120.8
26	51	25	96.2	22	45	23	104.6	22	48	26	118.2
24	53	29	120.8	27	56	29	107.4	23	49	26	113.0
29	54	25	86.2	27	56	29	107.4	26	51	25	96.2
23	48	25	108.7	30	62	32	106.7	23	47	24	104.3
30	55	25	83.3	27	45	18	66.7	29	53	24	82.8
25	54	29	116.0	26	52	26	100.0	22	52	30	136.4
21	43	22	104.8	26	50	24	92.3	30	57	27	90.0
29	58	29	100.0	26	53	27	103.8	26	53	27	103.8
25	52	27	108.0	21	39	18	85.7	22	48	26	118.2
22	48	26	118.2	26	52	26	100.0	21	49	28	133.3
21	43	22	104.8	22	51	29	131.8	22	46	24	109.1
24	48	24	100.0	24	51	27	112.5	22	51	28	127.3
24	47	23	95.8	22	36	14	63.6	22	51	28	127.3
21	53	32	152.4	22	50	28	127.3	24	54	30	125.0
26	52	26	100.0	23	52	29	126.1	25	59	34	136.0
24	45	21	87.5	26	49	23	88.5	30	62	32	106.7
24	46	22	91.7	27	51	24	88.9	24	52	28	116.7
28	53	25	89.3	23	45	22	95.7	21	49	28	133.3
27	51	24	88.9	22	47	25	113.6	26	55	29	111.5
23	39	16	69.6	30	68	38	126.7	24	46	22	91.7
26	54	28	107.7	27	50	23	85.2	26	51	25	96.2
23	46	23	100.0	28	40	12	42.9	27	57	30	111.1
27	54	27	100.0	26	57	31	119.2	23	51	28	121.7
23	42	19	82.6	24	49	25	104.2	30	62	32	106.7
26	42	26	100.0	26	44	18	69.2	28	49	21	75.0
28	51	23	82.1	25	48	23	92.0	25	61	36	144.0
27	57	30	111.1	23	50	27	117.4	22	46	24	109.1
30	55	25	83.3	29	54	25	86.2	27	53	26	96.3
23	46	23	100.0	24	55	31	129.2	28	58	30	107.1
29	61	32	110.3	23	46	23	100.0	25	52	27	108.0
21	47	26	123.8	30	59	29	96.7	30	62	32	106.7
26	49	23	88.5	30	61	31	103.3	24	56	32	133.3
30	53	23	76.7	24	54	30	125.0	30	63	33	110.0
Mean Growth Increase, %			99.3				101.6				112.1
Standard Deviation			15.9				19.6				15.3

TABLE XIV

EFFECT OF HIGH ABS CONCENTRATION ON GERMINATION OF *Lupinus* SEEDS

0 mg/l ABS				5 mg/l ABS				10 mg/l ABS				20 mg/l ABS			
Length of Hypocotyl (mm)			Growth Increase (%)	Length of Hypocotyl (mm)			Growth Increase (%)	Length of Hypocotyl (mm)			Growth Increase (%)	Length of Hypocotyl (mm)			Growth Increase (%)
Initial	Final	Growth		Initial	Final	Growth		Initial	Final	Growth		Initial	Final	Growth	
25	47	22	88.0	22	48	26	118.8	25	41	16	64.0	22	46	24	109.1
24	42	18	75.0	22	47	25	113.6	22	39	17	77.3	27	51	24	88.9
29	53	24	82.8	28	51	23	82.1	26	44	18	69.2	27	52	25	92.6
21	44	23	109.5	23	50	27	117.4	24	42	18	75.0	21	39	18	85.7
28	54	26	92.9	25	49	24	96.0	25	39	14	56.0	25	50	25	100.0
24	44	20	83.3	27	51	24	88.9	28	49	21	75.0	26	48	22	84.6
23	42	19	82.6	22	39	17	77.3	22	37	15	68.2	23	44	21	109.5
27	52	25	92.6	24	40	16	66.7	24	46	22	91.7	25	50	25	100.0
29	54	25	86.2	24	45	21	87.5	27	51	24	88.9	25	46	21	84.0
26	46	20	76.9	30	49	19	63.3	23	41	18	78.3	27	54	27	100.0
27	51	24	88.9	21	41	20	95.2	23	46	23	100.0	22	43	21	95.5
22	39	17	77.3	24	41	17	70.8	25	46	21	84.0	22	46	24	109.1
25	44	19	76.0	27	54	27	100.0	26	51	23	88.1	30	55	25	83.3
22	43	21	95.5	25	46	21	84.0	23	44	21	91.3	24	45	21	87.5
22	42	20	90.9	25	49	24	96.0	25	45	20	80.0	24	46	22	91.7
26	49	23	88.5	21	39	18	85.7	23	50	27	117.4	24	50	26	108.3
21	36	15	71.4	22	46	24	109.1	27	54	25	92.6	25	54	29	116.0
30	54	24	80.0	25	48	23	92.0	23	48	25	108.7	26	52	26	100.0
21	36	15	71.4	30	55	25	83.3	21	43	22	104.8	26	54	28	107.7
23	47	24	104.3	29	59	30	103.4	24	47	23	95.8	21	47	26	123.8
25	39	14	56.0	22	48	26	118.2	23	51	28	121.7	29	54	25	86.2
27	45	18	66.7	22	42	20	90.9	24	50	26	108.3	26	52	26	100.0
21	36	15	71.4	23	44	21	91.3	25	46	21	84.0	21	42	21	100.0
29	53	24	82.8	24	47	23	95.8	26	49	23	88.5	24	47	23	95.8
28	47	19	67.9	22	50	28	127.3	21	42	21	100.0	24	50	26	108.3
26	41	15	57.7	26	54	28	107.7	26	51	25	96.2	23	48	25	108.7
21	36	15	71.4	25	47	22	88.0	25	48	23	92.0	25	49	24	96.0
30	52	22	73.3	27	49	22	81.5	28	49	21	75.0	26	54	28	107.7
21	39	18	85.7	24	51	27	112.5	22	44	22	100.0	23	42	19	82.6
26	47	21	80.8	28	44	16	57.1	28	54	26	92.9	24	48	24	100.0
23	42	19	82.6	27	46	19	70.4	22	49	27	122.7	30	53	23	76.7
21	37	16	76.2	30	59	29	96.7	26	50	24	92.3	25	56	31	124.0
27	46	19	70.4	21	38	17	81.0	29	52	23	79.3	25	52	27	108.0
24	43	19	79.2	24	52	28	116.7	22	48	26	118.2	27	53	26	96.3
25	43	18	72.0	23	47	24	104.3					25	50	25	100.0
Mean Increase, %			80.2				93.4				90.3				99.1

100 mg/l ABS and above there is a continuous decrease in seedling weight and water content demonstrating an inhibitory effect at these concentrations.

TABLE XV

EFFECT OF ABS ON GERMINATION OF CORN SEEDS

ABS (mg/l)	Mean Weight (g)		Water Content Fresh Wt. Basis (%)
	Fresh	Dry	
0	0.50	0.24	73.3
25	.47	.21	74.9
50	.48	.22	74.7
100	.38	.17	72.3
200	.31	.17	70.1
500	.23	.15	65.1

IV. DISCUSSION

ABS IN SOILS

It has been observed that neither continuous percolation nor prolonged periods of static contact are conducive to ABS degradation, but a regular schedule of dosing with frequent, short rest periods gives maximum ABS breakdown. Since degradation of ABS in soils is dependent on aerobic biological activity, optimal conditions exist when an ample supply of substrate and sufficient oxygen for bacterial growth are available. Therefore, a balance must be struck between the rest periods necessary for proper soil aeration and the doses of sewage effluent requisite for food supply.

Another factor to be considered is loading rate. In the experiments reported, ABS degradation was increased from 25 to 35 per cent by reducing the loading rate from 1.10 to 0.29 mil gal/acre/day. To what extent ABS degradation may be maximized depends on land availability in a given sewage spreading operation and on the development of an optimum operational procedure.

ABS IN PLANTS

The capabilities for uptake of ABS by growing plants was demonstrated in both water culture and soil studies. ABS concentration in plant structure increased as the ABS concentration of the irrigation water increased. However, this relationship was not proportional except in one instance (barley growth in water culture). In one study (sunflower growth in water culture) the plant ABS concentration was several-fold greater than a proportional increase, and in another study (sunflower in soil) the relationship was less than a proportional increase. No conclusion could be drawn from barley grown in soil since it was analyzed for content of ABS in the grain only and the trace amounts found to be present were insufficient for valid correlation. A correlation could be made by using the concentration of radiosulfur present in the grain which was considerably higher than its ABS concentration. With two exceptions (5 and 15 mg/l ABS), there was a general trend of increasing S^{35} concentration in the grain as water ABS concentration increased, but this increase was less than proportional. In settled sewage irrigation the increase was continuous. A great deal more information would be necessary before conclusions could be drawn on the factors affecting ABS uptake. Whether the presence of other ions interfere in rate of uptake is not clear, but it is beyond the scope of this investigation to make a full-scale study of these factors. Generally speaking, soil studies showed that the uptake of ABS by plants tended to rise significantly but less than proportionally in relation to an increase in irrigation water ABS concentration.

An important aspect in regard to plant ABS uptake is the reduction of soil ABS content. It was found that the stems and leaves of sunflowers irrigated with sewage removed 3.3 per cent of the ABS adsorbed on the soil in the root zone. A conservative estimate of the root ABS concentration at twice that amount, based on the water-culture studies in which roots were analyzed, would mean a total reduction in soil ABS content on the order of 10 per cent in the root zone. Transfer of ABS from soil to plants is advantageous in lessening ground water contamination in two ways. The soil capacity for adsorbing ABS is increased, and the easily leached soil ABS is placed in a relatively fixed position in the plant structure.

Although the presence of ABS was detrimental to plant growth in some instances (particularly in water culture), in soil studies plants irrigated with sewage containing 4.6 to 12.7 mg/l ABS flourished by comparison to those irrigated with ABS-free water, regardless of soil fertilization practices. Only one species (sunflower) of the three grown in soil exhibited toxicity reactions to ABS (at concentrations of 10 mg/l and above), and this occurred under conditions of water irrigation of a nutrient-deficient soil. Barley and Lupinus albus irrigated with water and grown in fertilized soil showed no toxic response to ABS.

In view of these results it is likely that ABS is only toxic to plants grown in soil lacking the proper nutrients. In field-scale sewage spreading operations, where nutrient deficiencies could hardly be expected to occur, there should be no problem with ABS toxicity. In fact, the results show conclusively that irrigation with sewage effluent is beneficial to plants. Both barley and sunflower, when irrigated with sewage, surpassed their controls irrigated with ABS-free water in terms of plant height, weight, and general appearance. In the case of barley, the results were particularly meaningful because the soil was fertilized. It may be concluded then that the toxic effect of ABS on plants is far outweighed by the beneficial effects of the nutrients in sewage.

In view of the foregoing considerations, it is interesting to speculate why the toxicity of ABS to plants was so marked in the water culture studies. Nutrient deficiency could not be the cause, as it apparently was in the soil studies. It therefore appears that although water culture is a reliable method for testing most pharmacological products, a unique situation must exist in the case of surface active agents. Possibly interference in oxygen uptake by the roots is a cause, since it is known that surface active agents reduce oxygen transfer. Since little is known of the precise mechanism by which roots take up water and nutrients, no conclusions can be made regarding the root-water-ABS relationship compared with the root-soil-ABS relationship in plants.

It is too early to evaluate the practical merits of further experiments on the phenomena associated with ABS uptake by plants. Other considerations of water pollution have intensified a search for economical detergents having different characteristics than ABS. Should such compounds come into use experimentation on soil-plant-detergent relationships will certainly be undertaken.

V. SUMMARY AND CONCLUSIONS

The results of investigations conducted by the Sanitary Engineering Research Laboratory of the University of California during the second year of a projected three-year study of the fate of detergents in percolating waters are reported.

The majority of the work dealt with the effect of ABS on plant growth, and the uptake of ABS by plants. Two species of plants (sunflower and barley) were grown in water culture at ABS concentrations of 0, 10, and 40 mg/l, and three species (sunflower, barley, and Lupinus albus) were grown in soil at ABS concentrations up to 50 mg/l. In soil studies water solutions of ABS were compared to primary sewage effluents containing added amounts of ABS on both fertilized and unfertilized soils. Corn and Lupinus albus seeds were germinated in the presence of various concentrations of ABS to determine ABS effects at this stage of growth.

The two major findings of the studies on plants were that although ABS caused severe growth inhibition in water culture studies (about 70 per cent at 10 mg/l ABS and almost 100 per cent at 40 mg/l), only one species (sunflower) of the three species grown in soil was adversely affected. Furthermore, plants irrigated with sewage containing 4.6 to 12.7 mg/l ABS far surpassed in growth those irrigated with water, regardless of soil fertilization practices or the addition of up to 15 mg/l ABS to sewage. It may be concluded, therefore, that irrigation with sewage is beneficial to plants in spite of the presence of ABS in any amount likely to occur in sewage at the present time.

Other notable findings of the plant studies are as follows:

1. In water culture studies, ABS concentrated to a considerable degree in plant roots (up to 9 per cent in sunflowers and 2 per cent in barley on a dry weight basis) and to a lesser extent in stems and leaves (up to 0.2 per cent).

2. In water culture, degradation products of ABS were present primarily in the stems and leaves (up to 0.2 per cent) with only traces present in the roots.

3. Chlorosis of the leaves was evident in water culture studies --particularly in sunflowers grown in 10 mg/l ABS.

4. In soil studies, sunflowers grown at 5 mg/l ABS were unaffected, but those at 10 mg/l were 30 per cent retarded and at 50 mg/l were 65 per cent retarded.

5. Sunflowers receiving primary sewage effluent (containing 4.6 to 12.7 mg/l ABS) flourished in comparison to control plants receiving tap water and grown in unfertilized soil (7-fold increase in mean fresh weight).

6. Concentration of ABS in sunflowers grown in soil was small (less than 0.01 per cent in the stems and leaves), and ABS degradation products were present in slightly greater concentrations (0.02 to 0.07 per cent in the stems and leaves).

7. It was calculated that approximately 10 per cent of the final ABS content of the soil was taken up by sunflowers (including estimated root ABS concentration) irrigated with sewage effluent.

8. In soil studies the only plants to be affected by ABS were sunflowers grown in unfertilized soil. The other two species (Lupinus albus and barley), grown in fertilized soil, showed no sensitivity to ABS at concentrations up to 20 mg/l. It may then be true that plant growth is retarded by ABS only in a nutrient-deficient soil.

9. Only a trace (less than 0.001 per cent) of ABS was found in the edible portion of barley irrigated with up to 25 mg/l ABS. Degraded ABS was found in concentrations on the order of 0.01 per cent.

10. In the seed germination stage Lupinus albus was increasingly stimulated by ABS in concentrations ranging from 5 to 20 mg/l. Corn seeds were increasingly retarded by ABS at concentrations above 25 mg/l.

Experiments concerned with maximizing the ABS-degradation capabilities of soils in the biologically-active surface zone were continued from the first year. Laboratory-scale column studies were carried out on feed solutions equivalent to a secondary-sewage effluent containing approximately 20 mg/l BOD, as well as ions normal to sewage effluents. ABS concentrations of the order of 1 mg/l were traced with ABS^{35}. These studies support the following conclusions:

1. Maximum ABS degradation occurs when percolation is intermittent (at a loading rate of 1.1 mgd/acre ABS degradation averaged 25 per cent; and at 0.29 mgd/acre, 35 per cent).

2. Small doses applied at frequent, regular intervals enhance ABS degradation in contrast to large doses applied infrequently (in the latter case, reported in the First Annual Report, only 3.8 per cent ABS degradation occurred at a loading rate of 0.12 million gallons per acre per day).

3. Continuous percolation through a coarse soil overlaid by a finer soil gives substantial ABS degradation (20.5 per cent at a loading rate of 0.65 million gallons per acre per day).

4. Although ABS degradation may be improved by the aforementioned loading factors, such degradation is insufficient to prevent contamination of ground water.

APPENDIX A

NUTRIENT PREPARATIONS

PREPARATION OF WATER CULTURE

1. Prepare molar stock solutions of the following and use the amounts indicated per liter of nutrient solution:

Compound	ml/l
M KH_2PO_4	1
M KNO_3	5
M $Ca(NO_3)_2$	5
M Mg SO_4	2
Micronutrient solution	1

2. Micronutrient solution:

Compound	g/l
H_3BO_3	2.86
$MnCl_2 \cdot 4\ H_2O$	91
$ZnSO_4 \cdot 7\ H_2O$	0.22
$CuSO_4 \cdot 5\ H_2O$	0.08
$H_2MoO_4 \cdot H_2O$ (Assaying 85 per cent MoO_3)	0.02

3. Add iron in the form of 0.5 per cent iron citrate at the rate of 1 ml/l about twice a week or as indicated by appearance of plants.

4. Use di tilled water in all solutions and adjust pH of nutrient solution to 6 by adding 0.1N H_2SO_4.

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FERTILIZATION OF SOIL

1. Prepare air-dried soil by pulverizing and thorough mixing. If necessary, pass through a half-inch mesh sieve.

2. Add an underdrain to each pot using a piece of pyrex glass to cover the hole, then add quarter-inch layers of Fairoaks pea gravel, Monterey coarse and Monterey fine sand.

3. Add 1600 g of soil to each pot.

4. Add the following compounds to each pot:

Compound	Grams	Equivalent to:
NH_4NO_3	0.457	200 lb N per acre
$Ca(H_2PO_4)_2 \cdot H_2O$	0.426	300 lb P_2O_5 per acre
K_2SO_4	0.296	200 lb K_2O per acre

HOAGLAND'S SOLUTION

Prepare one-quarter strength Hoagland's solution as follows:

Stock Solution	ml Stock Solution per Liter of Nutrient Solution
0.5 M KNO_3	2.5
0.5 M KH_2PO_4	0.5
0.5 M $Mg\ SO_4$	2.0
0.5 M $Ca\ (NO_3)_2$	3.6

APPENDIX B

SELF-ADSORPTION

SUNFLOWER

1. Grind the stems and leaves and roots to a fine powder and weigh into 600 ml beakers in amounts ranging from 200-1000 mg.

2. Add 100 ml of ABS^{35} solution at an activity level of 464 cpm/ml to each beaker, bring to dryness on a steam bath, oven-dry for 1 hour and desiccate.

3. Powder the dried material, plate out (infinite thickness), and count.

4. S^{35} adsorption will be as follows:

a. Stems and Leaves

Wt. Dried Sample (mg)	cpm	Self-Adsorption Factor (for 500-mg Material)
203.4	1970	57.9
485.3	829	57.7
748.7	534	58.0
981.4	421	56.1
	Average	57.4

b. Roots

Wt. Dried Sample (mg)	cpm	Self-Adsorption Factor (for 500-mg Material)
210.2	1891	58.4
479.2	759	63.8
890.0	469	55.6
	Average	59.3

5. Calculation of self-adsorption will be as follows:

   Self-adsorption factor of 1:1 mixture (Carbon S.A. = 50 at 50 mg)

   $$= \left[\left(\frac{687/2 - 315}{687}\right) \times 50 + 50\right] = 52 .$$

   Therefore,

   S^{35} self-adsorption factor of plant material (at 500 mg) = 54.

6. Adsorb ABS^{35} counts on a mixture composed primarily of carbon, retaining only a small quantity of plant material in the ether phase during extraction.

7. Calculate self-adsorption of mixture based on carbon S.A. of 50 and plant S.A. of 54 at 500 mg.

   Sample calculation:

   Mixture composed of 600 mg carbon, 20-mg plant material.

   Weight correction to 500 mg: $\text{cpm} \cdot \frac{600 + 200}{500}$.

   Proportion of mixture at known self-adsorptions:

   $$.75(50) + .25(54) .$$

   Therefore,

   Self-adsorption correction (at standard 500-mg weight)

   $$= \text{cpm} \cdot \frac{800}{500} \left[.75(50) + .25(54)\right] .$$

BARLEY (GRAIN)

1. Harden barley for two weeks before harvesting.

2. Separate the grain and oven-dry at 105°C for 20 minutes.

3. Mechanically mill to a fine powder.

4. Mix powder with activated carbon of a known S^{35} activity and count the mixture.

5. After obtaining S^{35} counts return material to beakers, add 100 ml of 70% ethanol, and bring to dryness on steam bath.

6. Perform ether extraction for ABS^{35} [1] and plate out dried solids (infinite thickness).

7. ABS^{35} adsorption will be as follows:

   a. Stems and Leaves

Wt. Dried Sample (mg)	cpm	Self-Adsorption Factor (for 500-mg Material)
505.5	705	65.0
249.0	1370	67.9
	Average	66.5

   b. Roots

Wt. Dried Sample (mg)	cpm	Self-Adsorption Factor (for 500-mg Material)
452.9	682	68.0
222.3	605	76.7
	Average	72.4

BARLEY (STEMS, LEAVES, AND ROOTS)

1. Dry the plant material in an oven at 105°C for one hour, desiccate, and powder.

2. Take carbon of known activity (687 cpm in a standard planchet) and make 1:1 mixture with plant material.

3. Plate mixtures and count at infinite thickness.

4. S^{35} counts will be as follows:

   a. Root:carbon mixture = 315 cpm

   b. Stem and leaf:carbon mixture = 315 cpm.

5. Calculate self-adsorption factors:

carbon = 695 cpm

grain = 10 cpm

Mixture of 10% carbon and 90% grain = 119 cpm .

Assuming grain to be equal to carbon in self-adsorption:

10% carbon cpm = 0.10 (695) + 0.90 (10) = 79

Barley S.A. = $\frac{119-79}{695}$ x 100 = 5.8% less than carbon S.A.

Carbon S.A. = 50 (at 500 mg)

Barley S.A. = 50 (.942) = 47 (at 500 mg) .

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